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Osthole: A Multifunctional Natural Compound with Potential Anticancer, Antioxidant and Anti-inflammatory Activities

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Abstract

Nature has always proved to be a significant reservoir of bioactive scaffolds which have been used for the discovery of drugs since times. Medicinal plants continue to be a solid niche for biologically active and therapeutically effective chemical entities, opening up new avenues for the successful treatment of several human diseases. Contribution of plant-derived compounds either in their original or semi-synthetic derivative forms extends far back in time in drug discovery. This review aims to focus on the sources, biological, and pharmacological profile of a pharmacologically active plant-derived coumarin, osthole, which is an important component of numerous remedial plants such as *Cnidium monnieri*. Several studies have revealed that osthole possess pharmacological properties such as anticancer, antioxidant, anti-hyperglycemic, neuroprotective, and antiplatelet. Osthole has been reported to regulate various signaling pathways which in turn modulate several apoptosis related proteins, cell cycle regulators, protein kinases, transcriptional factors, cytokines, and growth receptors affiliated with inflammation, proliferation and several other ailments. Osthole is known to halt proliferation and metastasis of cancerous cells by arresting the cell cycle and inducing apoptosis. The data in this review paper supports the pharmacological potential of osthole but further experimentation, biosafety profiling and synergistic effects of this compound need to be focused by the researchers to understand the full spectrum of pharmacological potential of this therapeutically potent compound.

Keywords: Osthole, anti-inflammatory, antioxidant, anticancer, neuroprotective

1. Introduction

Natural products have been utilized as therapeutic agents and act as a valuable source to invent new novel drug leads [1]. Various diseases have been cured by using the medicinal plants since prehistoric times [2-4]. “Natural products” are generally referred to as chemical entities with the origins from living organisms e.g., plants, microorganisms and marine organisms [5, 6]. Medicinal plants containing a vast range of phytochemicals including phenolics (flavonoids, coumarins, quinones, lignans, tannins, terpenoids, and stilbenes), carotenoids, β-carbolines (betalains, alkaloids), vitamins, and endogenous antioxidant metabolites have been reported to possess scavenging properties against free radicals [7-14]. Certain investigations have revealed that these bioactive chemical entities exhibit pharmacological properties against inflammation, tumor progression, mutagenesis, atherosclerosis, bacteria, and viruses to somewhat more or lesser extent [15-22].

Cnidium monnieri (L.), an important Chinese medicinal herb, from the plant family Umbelliferae is natively distributed in China, Russia, Korea and Mongolia. The dried fruits of this plant are used all over the China for curing male impotence, swelling of women's genitalia and blood stasis. The useful effects of this medicinal herb are recommended due to presence of numerous coumarin compounds [23]. Coumarins (2H-1-benzopyran-2-one) constitute a vast class of polyphenols identified in many plants, and microorganisms. This class of compounds is famous for their potential anticancer, neuroprotective, antioxidant, anti-inflammatory, antihyperglycemic, and antibacterial properties [24]. Osthole (7-methoxy-8-(3-methyl-2-butenyl)-coumarin) has caught attention in recent years due to its wide range of pharmacological and biological characteristics [25-28].

Accumulated data suggested that osthole exhibits distinct pharmacological traits including antiplatelet [29], anti-allergic [30, 31], reduction of bone loss in ovariectomized (OVX) rats [32], inhibition of systolic blood pressure [33], vasorelaxant [34], anti-inflammatory [35], anti-osteoporotic [36, 37], and anticancer [38-41], [42, 43].

The data presented in this article was collected from different electronic sites i.e., Scopus, PubMed, Sci-hub, Google Scholars, and Elsevier Science Direct. We used “osthole”, “osthole and biological activity”, “anticancer activity”, “anti-inflammatory activity”, “antioxidant”, and “neuroprotective” as key words for searching.

2. Natural sources of osthole

Osthole (Figure 1), a natural coumarin, has commonly been isolated from the plants of Apiaceae family such as *Angelica genuflexa* [44] and *Angelica japonica* [45]. Besides them, osthole has been reported in *Arracacia tolucensis var. multifida* [46], *Atractylodes lancea* [47] and *Citrus aurantium* [48]. Another rich source of osthole is *C. monnieri* [49-55] which has a number of pharmacological activities including anticancer, anti-larval, anti-inflammatory, anti-diabetic, anti-hypertensive, neuroprotective, anti-osteoporosis, hyperlipidemic, anti-viral, antioxidant, antihelmintic, hepatoprotective, anti-allergic, antipruritic and vasorelaxing effects [31, 49-83]. As shown in Figure 1, osthole has also been isolated from *Clausena guillauminii* [71], *Clausena lansium* [84], *Euphorbia fischeriana* [85], *Ferulago capillaris/ brachyloba* [86], *Fructus psoralea* [87], *Heracleum rapula* [88], *Imperatoria osthruthium* [89], *Libanotis buchtormensis* [90], *Peucedanum ostruthium* [37, 91, 92], *Prangos ferulacea* [93, 94], *Radix angelicae pubescentis* [29, 62, 64, 95-103], *Siegesbeckiae herba* [104], *Seseli gummiferum* [105], and *Skimmia anquetelia* [106]. Table 1 reprsents biological sources of osthole and the well known therpaeutic properties attributed to these medicinal plants.

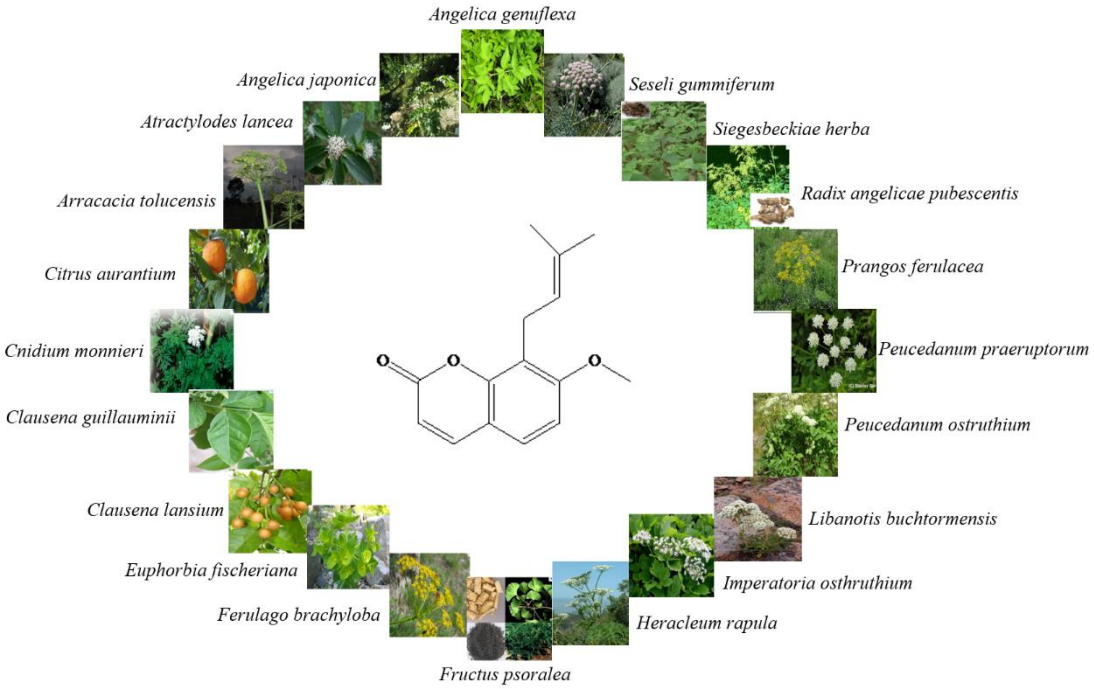

Figure 1. Chemical structure of bioactive coumarin, osthole, and its biological sources

Table 1. Biological sources of osthole with their various pharmacological properties.

Plants			Parts used/ extract	Pharmacological activities	References
Scientific Name	**Common Name**	**Native Name**			
Angelica genuflexa	Kneeling angelica	Qu Xi Dang Gui	Roots	Antiplatelet, anti-coagulant	[44]
Angelica japonica	Japanese angelica	Ri Ben Dang Gui	Roots	Antiproliferative	[45]
Arracacia tolucensis var. multifidi	-	-	Seeds (oils)	Antimycobacterial	[46]
Atractylodes lancea	-	Cangzhu	Rhizome	Anti-inflammatory	[47]
Citrus aurantium	Bitter orange	Zhi Shi	Fruits	Antibacterial, antifungal	[48]
Cnidium monnieri	Fructus cnidii	She Chuang Zi	Seeds, fruits	Antitumor, improves spatial memory deficits, anti-larval, anti-inflammatory, improves sexual dysfunction, anti-diabetes, anti-hypertensive, regulation of cardiac oxidative stress, neuroprotective, anti-osteoporosis, anti hyperlipidemic, anti-viral, antioxidant, antihelmintic, hepatoprotective, anti-allergic, antipruritic, vasorelaxant effect	[49-55]
Clausena guillauminii	-	-	-	Anti-inflammatory	[71]
Clausena lansium	Wampee	Shí Huáng Pí	Roots	Anti-inflammatory	[84]
Euphorbia fischeriana	Lang du	Láng Dú Dà Jǐ	Roots	Antitumor	[85]
Ferulago capillaris/ brachyloba	-		Roots	-	[86]
Psoralea corylifolia	-	Bu Gu Zhi	Seeds	Antitumor	[87]
Heracleum rapula	White cowparsnp	Bai Yun Hua	Roots	-	[88]
Imperatoria osthruthium	Masterwort	-	Seeds	-	[89]
Libanotis buchtormensis	-	Yan Feng	Supercritical extract	Analgesic, anti-inflammatory	[90]
Peucedanum osthruthium	Masterwort	Ou Qian Hu	Rhizomes	-	[92]
Prangos ferulacea	Ribbed cachrys	-	Roots	Antispasmodic	[93, 94]
Radix angelicae pubescentis	Hairy angelica root	Du Huo	Roots	Anti-inflammatory, pulmonary vasodilator, anti-diabetic, anthelmintic, vasorelaxant effect	[29, 62, 64, 95-103]
Selinum cryptotaenium	-	Liang She Chuang	Roots	-	[107]
Siegesbeckiae herba	Siegesbeckiae	Xi-Xian	Aerial parts	-	[104]

Seseli gummiferum	Moon carrot	-	Aerial parts	Anti-inflammatory, antinociceptive	[105]
Skimmia anquetelia	Magic marlot	-	Leaves	Antibacterial	[106]

3. Biological activities of osthole

Osthole has been documented to possess versatile range of biological as well as pharmacological properties including anticancer, antioxidant, anti-diabetic, anti-inflammatory, neuroprotective, anti-osteoporosis, vasorelaxant, and anti-thrombotic as depicted by Figure 2. Several experiments (*in vivo* as well as *in vitro*) have highlighted the pharmaceutical potential and action mechanisms of this bioactive compound.

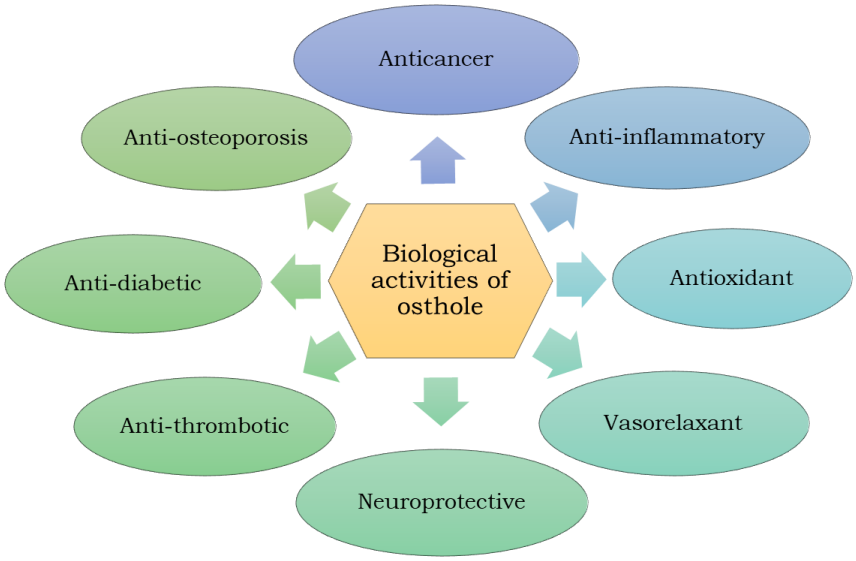

Figure 2. Biological activities of osthole

3.1. Osthole and its anti-inflammatory activities

The immediate defensive response of a living body against infection or pathogenic invasion in order to restore the homeostatic balance is termed as inflammation. When it happens in an unrestrained or inappropriate manner, it can cause the development of certain physiological malfunctioning incorporating cancer and many neurodegenerative diseases [108]. This uncontrolled inflammatory effect is then activated by an external bacterial endotoxin, lipopolysaccharide (LPS), which results in the stimulation of different cytokines e.g., tumor necrosis factor, interleukins, MMPs, NO, eicosanoids, and other inflammatory mediators [71].

Osthole, a natural bioactive coumarin, induces anti-inflammatory effects in various animal models as summarized in Table 2. Murine macrophage (RAW 264.7) cells, when treated with osthole, effectively inhibited inflammatory mediators such as IL-6, iNOS, TNF-α, NO, and COX-2. Osthole also decreased the activity of angiotensin-converting enzyme 2 (ACE2) as well as increased the activity of TRX1 (thioredoxin 1) and nuclear factor-like 2 (Nrf2) in LPS-stimulated acute lung injury [56, 71, 109]. In addition to that, osthole prohibited phosphorylation of p38 MAPK and PKC-α along with the suppression of NF-κB in LPS-treated macrophages [110].

Furthermore, osthole has potential to down-regulate the levels of myeloperoxidase (MPO), IL-1β and IL-8 when treated with MCAO rat model of acute ischemic stroke [111]. Moreover, administration of osthole significantly inhibited albuminuria and ameliorated renal system functions including glomerular growth and periglomerular mononuclear leukocyte infiltration in IgAN-progressive mice model. The associated mechanism involves reduction in MCP-1, superoxide anion levels, ROS inhibition and NF-κB activation [112].

In another study, osthole has been reported to be defensive against renal (I/R) damage as the mechanism of action of this bioactive coumarin was linked with the down-regulation of NF-κB signaling, TNF-α, IL-6, and IL-8 levels [113] while p38 MAPK expression was activated in the kidneys [9]. Further, osthole induced attenuation of hepatic injury via decreasing myeloperoxidase activity, interleukin-6 and ICAM-1 levels along with the increase in p38 MAPK phosphorylation [114]. Osthole possess promising capability to block lung inflammation on carrageenan-stimulated pleurisy in rats along with the inhibition of PMNs infiltration, reduction in MPO levels and release of inflammatory mediators such as IL-1β and TNF-α [115]. Moreover, osthole has been reported to reduce lipid accumulation via down-regulating triglyceride, total serum cholesterol, TNF-α, and malondialdehyde (MDA) levels as well as up-regulating GSH levels in the mice liver [116].

Osthole inhibits non-alcoholic fatty liver disease by reducing lipid augmentation via suppressing malondialdehyde concentration as well as NF-κB/p38 MAPK signaling pathways in FL83B cells [117]. It also showed potent anti-inflammatory effects in chronic kidney failure model of rat via decreasing NF-κB activity, TGF-β1 and monocyte chemoattractant protein-1 levels along with activation of PI3K/Akt/Nrf2 pathway [118]. In another investigation, osthole significantly

lowered RV pressure and ameliorated mitochondrial swelling, myocardial hypertrophy with sarcoplasmic reticulum amplification on MCT-mediated right ventricle (RV) rat remodeling which ultimately resulted in prohibition of myocardial inflammation [119]. Furthermore, 50 μM and 100 μM treatment of osthole blocked interleukin-1β-induced growth and migration of SW982 cells *in vitro* via inhibiting MMP-1/-3/-13, TNF-α and IL-6 levels along with suppressed expression of MAPK and NF-κB signaling cascades. It also improved collagen-mediated arthritis in a rat model *in vivo* [120]. Likewise, the protective effects of osthole were also observed in mouse microglial BV-2 cells. Results declared that osthole treatment inhibits LPS-stimulated secretion of inflammatory cytokines and activation of NF-κB signaling and increasing levels of HO-1 and Nrf2 in a dose-dependent way [121]. Pretreatment of osthole alleviated acetamophen-induced hepatocyte necrosis with increasing levels of ALT and AST activities along with the reduction in the inflammatory cytokines and cytochrome P450 enzymes. An increase in the expression of UGTs and SULTs was also noticed. Hence, we can conclude that osthole prevents acetamorphin-induced hepatotoxicity and exerts a preventive effect by increasing the antioxidant mechanism of liver [122]. Osthole elevated the levels of SOD2, GPX1, GCL-c, and G6pdh, and retarded the expression of AOCX and NOX2 [123]. Osthole treatment significantly reduced the secretions of TNF-α, IL-6 and IL-1β in BV2 cells stimulated by lipopolysaccharides [121].

Table 2. Brief outline of anti-inflammatory activities of osthole along with the molecular targets.

Assay	Organism tested	Dose/conc.	Molecular targets	References
Preventive effects of osthole on the progression of IgAN using Prg-IgAN model	Mice	50 μM	Activation of NF-κB$^{\perp}$, Nrf2↑, Activation of NLRP3$^{\perp}$, MCP-1↓, ROS$^{\perp}$	[112]
Impacts of osthole on hepatic injury	Rats	3 mg/kg	p38 MAPK↑, reestablished the expression of IL-6, MPO, ICAM-1	[114]
Effects of osthole on myocardial injury	Rats	1, 10 or 50 mg/kg	HMGB1↓, NF-κB↓	[124]
Effect of osthole on lung injury	Mice	40 mg/kg	Trx1↑, Nrf2↑, H_2O_2↓, MDA↓, OH↓	[56]
Effect of osthole on renal injury	Rat	40 mg/kg	MPO↓, IL-1β↓, TNF-α↓, p38 MAPK ^{Act}	[9]

Protective effect of osthole in lung injury in mice	Mice	40 mg/kg	TNF-α⊥, IL-6⊥	[109]
Antioxidant effects of osthole on middle cerebral artery occlusion (MCAO) on a rat model	Rats	30 mg/kg	Restoration of MCAO induced increased expression of IL-1β, COX-2, TNF-α, iNOS	[63]
Potential inflammatory activity of osthole in mouse macrophage	Macrophages	0.0086 ± 0.0024 μM	fMLP/CB-induced superoxide anion production⊥	[35]
Osthole effects on nucleus pulposus evoking nociceptive responses through down-regulation of over expression of ASIC3	Rats	-	ASIC3↓	[125]
Osthole neuroprotective effects including its potential mechanisms on middle cerebral artery occlusion in rats	Rats	10, 20 and 40 mg/kg	MPO↓, MDA↓, IL-1β↓, IL-8↓, GSH↑	[111]
Inhibitory effects of osthole on expression of inflammatory mediators in murine macrophage	Murine macrophage	1, 3 and 10 μg/mL	NO⊥, TNF-α⊥, COX-2⊥, p38⊥, p-JNK1/2⊥, p-PKC-α⊥, p-PKC-ε⊥, LPS-induced NF-κB activation⊥, ROS⊥	[110]
Efficiency of osthole on IL-4-induced exotoxin expression in BEAS-2B cells	Human (BEAS-2B cells)	0.1–10 μM	IL-4-induced STAT6↓	[126]
Potent inhibiting efficiency of osthole on the expression of inflammatory mediators in macrophages of mouse	Mouse (RAW 264.7) cells	10 μM, 5 μM	iNOS⊥, TNF-α⊥, NO⊥, COX-2⊥	[71]
Protective effects of osthole on lumber disc herniation induced sciatica	Rats	50 microL 2%	COX-2⊥, NOS⊥	[127]
Anti-inflammatory effects of osthole and its mechanism of action in rats	Rats	50, 100 mg/kg	PG↓, NO↓, MDA↓, cNOS↓, cGMP↑	[128]
Amelioration of inflammatory response by osthole in nonalcoholic steatohepatitic rats	Rats	20 mg/kg	MCP-1↓, TNF-α↓, IL-8↓, IL-6↓	[129]
Protective effects of osthole on oleic acid induced inflammation in FL83B cells	FL83B cells	-	p-AMPK↑, NF-κB↓, MAPK↓	[117]
Anti-inflammatory effects of osthole on chronic kidney failure in	Rat	40 mg/kg	TNF-α⊥, IL-6⊥, IL-8⊥, NF-κB↓, TGF-β1↓	[118]

rat modelf				
Attenuation of inflammation by osthole in monocrotaline-induced rat model	Rats	10, 20mg/kg	TNF-α↓, IL-6↓, p-NF-κB (p65)↓	[119]
Therapeutic effects of osthole on arthritis (*in vivo* and *in vitro*).	IL-1β-stimulated SW982 cells, collagen-induced arthritis rat model	20, 40 mg/kg (*in vivo*), 50, 100 μM (*in vitro*)	TNF-α⊥, IL-6 ⊥, MMP-1⊥, MMP-3⊥, MMP-13⊥, NF-κB⊥, MAPK⊥	[120]
Anti-inflammatory effects of osthole on LPS-induced inflammation in BV2 cells	BV2	10 μg/ml	NF-κB⊥, Nrf2⊥	[121]

↑Up-regulation; ↓Down-regulation; ⊥Inhibition; ^{Act}Activation; MPO: Myeloperoxidase; Trx1: Thioredoxin 1; HMGB 1: High mobility group box protein 1; p38 MAPK: p38 mitogen activated protein kinase; ACE2: Angiotensin converting enzyme 2; NO: Nitric oxide; TNF-α: Tumor necrosis factor-α; ASIC3: Acid sensing ion channel 3; STAT6: Signal transducer as well as activator of transcription 6; fMLP/CB: Formyl-l-methionyl-l-leucyl-l-phenylalanine/cytochalasin B

3.2. Osthole as neuroprotective agent

Various studies have reported that osthole also possesses neuroprotective potential. Osthole has pronounced ability to reduce cell viability losses, release of cytochrome c and LDH along with caspase-3 dose-dependently. It has also been involved in preserving the normal motor neurons by decreasing the levels of ROS. Osthole has potential capability to up-regulate Bax, activities of catalase, SOD, GSH, Erk1, and glutathione peroxidase (GPx) as well as production of adenosine triphosphate [130-132]. Osthole is involved in WNT/β-catenin activation which is further associated with the regulation of NSC proliferation and differentiation of neurons. This compound has also been documented as potent compound for the treatment of coronary occlusion-induced cerebral hypo-perfusion along with neural damage that can be acute especially in case of middle cerebral artery (MCA) in rats [133].

Furthermore, osthole has competency to reduce edema, MPO, infract volume, interleukin-1 beta (IL-1β), IL-8, and muscular dystrophy association (MDA) against severe strokes [111]. Moreover, myocardial I/R injury may also be cured by treatment of osthole as its mechanism of action proceeds via lowering the production of metabolites of lipid peroxidation, elevating

antioxidant abilities of enzymes, and preventing inflammation by inhibiting cytokines expression. In ischemic myocardial tissues osthole has a potential to reduce the expression of HMGBI along with NF-κB [124].

3.3. Osthole and its antioxidant activities

Byproducts of normal homeostasis and mitochondrial metabolism involve the accumulation of potentially harmful levels of ROS which needs to be formalized [134]. Imbalance among the production of ROS and cellular antioxidant defense systems results in oxidative stress. Oxidative stress is a causative factor for a variety of pathological conditions in living systems such as inflammation, endothelial dysfunction, angiogenesis, and hypertension [135].

Coumarins, a novel class of pharmacological agents, are known to strengthen the antioxidant defense systems [136]. Osthole, a naturally occurring coumarin, has promising potential to inhibit the generation of reactive oxidants such as superoxide anions [137]. This bioactive compound has competency to improve accelerated focal segmental glomerulosclerosis model at a very initial step via activation of Nrf2 with successive retardation in NF-κB-mediated cyclooxygenase-2 expression [138]. Accumulated data by researchers recommend that osthole has potent capability to ameliorate ischemia-reperfusion injuries in rats by suppressing oxidative stress and by strengthening antioxidant defense systems [124, 139-141]. The detail information regarding to effective inhibitory concentrations and molecular targets of osthole is provided in Table 3.

Table 3. Antioxidant activities of osthole and its action mechanism.

Activity	Organism tested	Dose/conc.	Mechanisms invloved	References
Protective neuroprotective potential of osthole against spinal cord ischemia-reperfusion injury	Rats	10 and 50 mg/kg	The water contents of spinal cord are decreased along with volume of infarct, ROS levels reduced, ATP generation enhanced with increase in mitochondrial respiratory chain complex activities	[131]
Effect of osthole on Aβ levels in neural cells and phosphorylation of CREB protein	Human (SH-SY5Y cell)	50 μM	Intracellular Aβ levels decreased in neural cells, BACE1 protein levels increased, reversed synapsin-1 reduction, reestablished phosphorylation of CREB protein	[142]
Osthole shows protective effects on middle cerebral artery	Rats	30 mg/kg	Restored the expression of MCAO induced increase in TNF-	[63]

occlusion (MCAO) model			α, IL-1β, COX-2, iNOS	
Neuroprotective role of osthole against ischemic injury (*in vitro*)	Rats	1, 10, 50 and 100 μM	Lowered JNK activity and increase in ERK1/2 activation	[53]
Neuroprotective potential of osthole on middle cerebral artery occlusion (MCAO) induced acute ischemic stroke in rats	Rats	10, 20 and 40 mg/kg	Increased GSH activity, decreased the volume of infarction, NDS, edema, MDA, MPO, IL-1β and IL-8	[111]
Protective effects of osthole on MPP (+)-induced cytotoxicity in PC12 cells	Rats	0.01, 0.05, 0.10 mM	Increased Bax/Bcl-2 ratio, reduced LDH release, decreased cytochrome c and caspase-3 activity, cell viability losses reduced	[68]
Effectiveness of osthole on chronic cerebral hypo-perfusion induced neuronal degeneration and cognitive impairment in hippocampus of rats	Rats	10, 20 mg/kg	Decreased production of malondialdehyde (MDA), decreased bax/bcl-2 ratio, increased activities of GPx and CAT	[130]
Protective effect of osthole and associated mechanism on learning and memory impairment in mice	Mice	15 and 7.5 $mg.kg^{-1}$	Enhanced activities of GSH-PX and SOD	[143]
Antioxidant effects of osthole in FL83B cells	FL83B cells	-	Blockage of NF-κB and MAPK signaling pathways	[117]
Efficacy of osthole against a rat model of hypoperfused retina	Rats	40 mg/kg	Reduction of NF-κB and Akt levels, Increased ratio of Bcl-2/Bax, Enhanced activities of SOD	[144]
Attenuation of type 2 diabetes in mice induced by streptozotocin and high-fat diet	Mice	20mg/kg	Increased insulin levels, enhanced activities of glutathione peroxidase, superoxide dismutase, catalase	[145]
Antioxidant effects of osthole against dinitrobenzene sulphonic acid induced-colitis in rats	Rats	50 mg/kg	Increased MDA and MPO, GSH, CAT and SOD levels	[146]

3.4. Osthole and its anticancer activities

Predestined and genetically modulated cell suicide activity is termed as apoptosis [147, 148]. Various complex signals are involved in this process e.g., DISC (Death-inducing signaling complex). DISC can promote DNA fragmentation, biochemical, and morphological aberrations such as membrane blebbing [149, 150].

Phytochemicals, from medicinal plants, are thought to be the most significant reservoirs for the discovery of anticancer drugs [151]. Studies have revealed that osthole shows synergetic effects on cell proliferation and tumor inhibition in different types of cancers.

Many studies on osthole have reported its antiproliferative traits because of its promising potential to induce apoptosis in different cancerous cells [152, 153] including prostatic cancer

(LNCaP, PC3, DU145) [154], leukemia (HL-60) [155], lung cancer cells (A549) [41] [156], breast cancer (MDA-MB 231, MCF-7) [69], hepatocellular cancer (HepG2, SK-LU-1) [54, 157, 158], epidermal carcinoma (KB) [54], human laryngeal cancer (RK33), medulloblastoma cell (TE671) [159], glioma cancer cells (U87) [160], colorectal cell line (CoLo 205) [155], nasopharyngeal carcinoma [161], esophageal [162], gastric [163], and colon cancer cells (HCT116, SW480) [164].

Osthole has potential capability to trigger apoptosis, to arrest cell cycle at S/G2 phases, to regulate TP53 gene expression and CDKN1A depending on cancer cells dose-dependently [159]. Treatment of hepatocellular cancer cell lines with osthole inhibited activity of NF-κB with the ultimate inhibition of tumor growth and induction of apoptosis [157]. Osthole's ability to prevent the multiplication of osteosarcoma (MG-63) cells is linked with the suppression of Bcl-2 while up-regulating Bax levels [165]. It can also inhibit the metastatic activity in lung adenocarcinoma cells (CL1-5, H1299) via inhibition of MMP-9 and IκBα degradation [166]. In case of glioblastoma multiforme (GBM), osthole blocked metastatic transformation occurs by inhibition of IGF-1-induced EMT along with blockage of PI3K/Akt pathway [167]. Osthole prohibited invasion and migration in AIPC cells *in vitro,* while metastasis of AIPC during *in vivo* studies in rats [168]. Osthole inhibited TGF-β/Smads signaling and regulated the OPG/RANKL signals in breast cancer cells [27].

Several investigations have documented that osthole is involved in arresting cell cycle at G1 phase in lung cancer cells [169] and in breast cancer (MDA-MB 435) cells via down-regulating Cdk2 and cyclin D1, along with the up-regulation of p21Waf1/Cip1 and p53 [170]. While in A549 cells, osthole induced arrest at G2/M phase by suppressing cyclin B1, Bcl-2 and p-Cdc2 expression, up-regulating the Bax expression and inhibiting PI3K/Akt signaling pathway [41]. Findings from an *in vitro* study has reported induction of apoptosis by osthole via suppressing NF-κB activity in liver cancer cells (Figure 3) [157].

3.4.1. Osthole and AMPK/PI3K/Akt and ERK Pathway

ERK (Extracellular signal regulated kinase) signaling cascade performs a significant function in the progression and development of tumors. Key factor of ERK pathway is ERK1/2, whose inhibition prevents tumor cell propagation. Phosphoinositide 3 kinase (PI3K)/Akt signaling network has also been associated with cell survival, proliferation and angiogenesis and is most

common signal transduction pathway activated during cancer [30, 171]. PI3K/Akt is also known to be a potential target for the cure of breast and several other cancer types [172]. These signaling pathways consist of several kinase cascades, which can be phosphorylated and de-phosphorylated by specific enzymes including phosphatases and adaptor proteins [30].

Natural compounds are known to specifically target various signaling pathways which perform an imperative function in cancer development and progression [169]. Treatment of osthole as an anticancer agent in A549 cells has declared the fact that it has significant potential to attenuate the phosphorylation of two crucial pro-survival kinases, ERK and Akt. Osthole is also involved in inhibiting the activation of AMPK which suggests that it can be a novel anticancer drug candidate for the treatment of lung cancer [173].

Osthole also has an ability to induce G2/M phase arrest and apoptosis in A549 cells via modulating the activity of PI3K/Akt signaling [41]. Phosphorylation of Akt, an important PI3K downstream target, was decreased in C6 cells after the administration of osthole [174]. Treatment of human brain cancer cells by osthole leads to the inhibition of IGF-1 stimulated EMT via blockage of PI3K/Akt pathway (Figure 3) [167]. During an *in vitro* investigation, osthole inhibited the proliferation of esophageal cancer cells via modulation of PTEN-PI3K/Akt signaling pathway [162]. In cholangiocarcinoma HCCC-9810 and RBE cancer cells, osthole down-regulated the expression of PI3K and phosphorylation level of Akt which contributed towards the osthole-induced apoptosis in these cells [175]. Thus, it is suggested that osthole has potential to induce mitochondrial-dependent apoptosis through PI3K/Akt signaling cascade [175].

3.4.2. Targeting NF-κB with osthole

NF-κB is widely accepted as an important player for cancer initiation and progression as it supplies oncogenic signals to tumor cells. Thus, this signaling cascade epitomizes a tempting target for cancer prevention [176].

It has been proved by multitudinous studies that osthole actively participates in the prevention of invasive and migratory capabilities of human lung CL1-5 cells by NF-κB pathway inhibition which can lead to the down-regulated MMP-9 levels [166]. Osthole has ability to suppress cellular proliferation in hepatocellular carcinoma cells via suppression of NF-κB in the dose-and

time-dependent manner [157]. Osthole was also noticed to reduce the cell viability, proliferation, migration, and invasion of cervical cancer cells dose dependently. It inhibited NF-κB activity while increasing the levels of E-cadherin and decreasing the levels of vimentin, thereby suggesting osthole as a therapeutic candidate for an adjuvant treatment for cervical cancer [177]

3.4.3. Targeting p53 with osthole

Mutated p53 gene is one of the crucial necessities for tumor initiation or development. Reviving the effectiveness of aberrant p53 through small molecules is an auspicious approach to combat cancer [178], [179, 180].

It has been reported that osthole is capable of inducing apoptosis and cell cycle arrest in breast cancer (MDA-MB 453) cells. The attempt to understand the underlying action mechanism of osthole has highlighted the fact that it significantly stimulates G1 phase arresting because of increasing activity of p21/p53 and reducing expression of cyclin D1 and Cdk2 (Figure 3) [170]. p53, a cell cycle regulatory protein, is a significant mediator for osthole-induced cancer prevention. Osthole has ability to activate p53 gene via up-regulating p53 phosphorylation on Ser15 (p-p53) and p53 acetylation on Lys379 (acetyl-p53) [164]. Osthole in HepG2 cells induced arrest at G2/M phase of cell cycle and ultimately triggered apoptosis by enhanced levels of pro-apoptotic Bax and p53 (Figure 3) [181].

3.4.4. Role of osthole in inhibition of tumor metastasis

One of the most fundamental challenges involved in curing cancer is tumor metastasis which is regarded as the transfer of tumorigenic cells from originative site to far-off organs for colonization [182, 183].

Matrix metalloproteinase-9 induction is specifically crucial for tumor metastasis in many cancers. Osthole, a pharmacologically active coumarin caused effective inhibition of the invasive ability of A549 cells via reduced expression of NF-κB mediated expression of MMP-9/-2 [166]. It has also been reported to possess suppressive effects on glioma cells (U87) proliferation via up-regulating the miR-16 expression and down-regulating the MMP-9 expression. As up-regulation of miR-16 can restrain tumor metastasis, therefore, osthole may has a potential as chemotherapeutic agent in future [160].

Osthole in combination with aconitine has capability to prohibit the migration of cancerous cells which is actually the result of alteration in the signaling pathway TGF-β/Smad along with decreased expression of NF-κB and RANK [184]. Data by researchers recommend that osthole has competency to inhibit the invasion as well as migration in human colorectal cancer cells by down-regulation of the transcription factor snail, MMP-9, and the vimentin. In contrast, it also has a potential to up-regulate the epithelial protein E-cadherin [185]. It is suggested that wenshen zhuanggu formula (WSZG) is capable of inhibiting invasiveness of tumor cells by suppression of TGF-βRI phosphorylation that can further hinder phosphatidylinositol 3-kinase/Akt signaling pathway and Smad2 [163, 186].

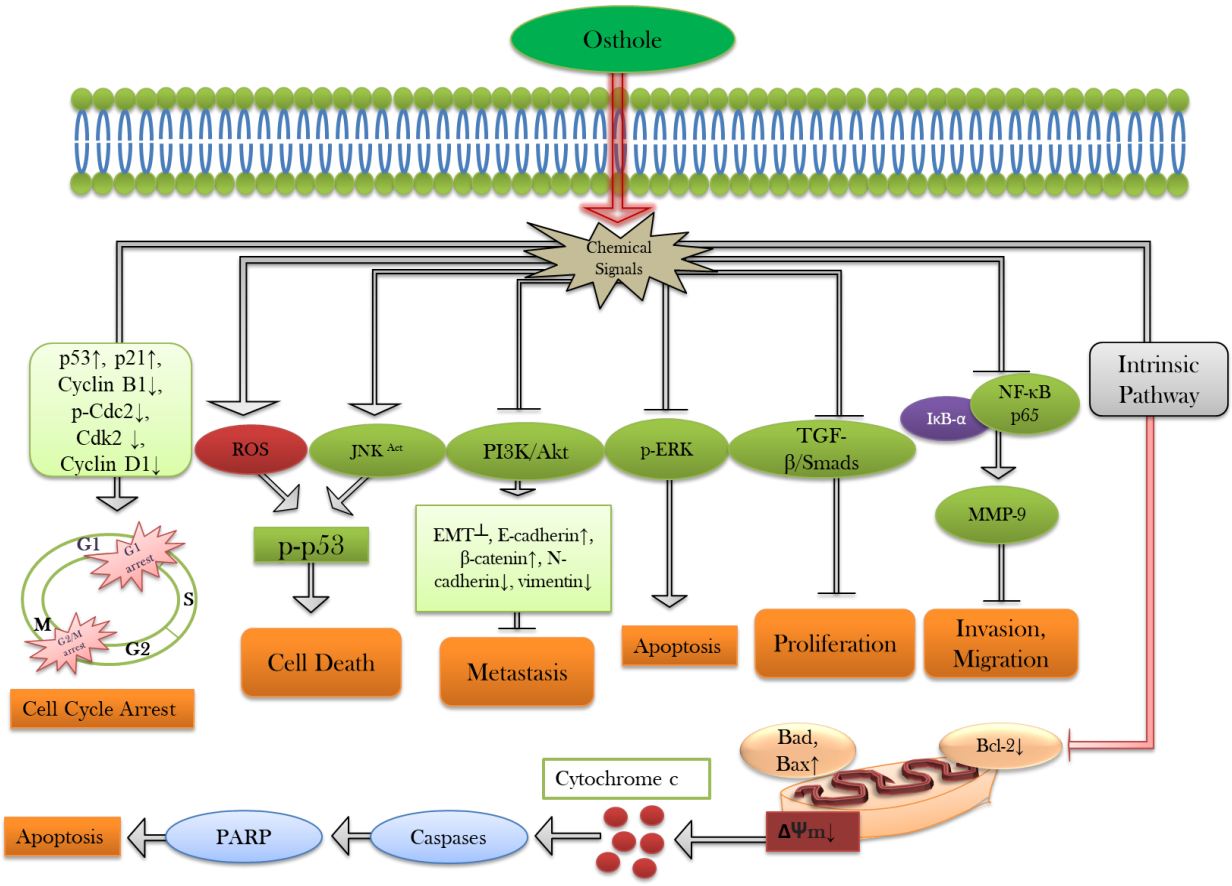

Figure 3. Diagrammatic representation of different molecular mechanisms responsible for the anticancer activity of osthole.

3.4.5. Micro RNA silencing

Micro RNAs are capable of modulating post transcription gene expression by hindering translation process or by enhancing the mRNA degradation [187, 188]. Micro RNA clusters

(miR-23a) are involved in regulating transforming growth factor-β1-induced EMT via targeting E-cadherin, thus, supporting tumorigenesis in cancer cells [7]. The study on profile of human cancer cells pinpointed a strong link among cancer metastasis and miRNA deregulation [189].

Osthole is capable of suppressing the miR-23a-3p expression as it has competency to suppress EMT-mediated invasive capability of AIPC by means of up-regulating the epigenetic EMT-related molecules; β-catenin, E-cad, and down-regulating the mesenchymal marker in prostate cancer cells DU145 and PC3 [168]. In glioma cells U87, osthole has been involved in the suppression of proliferation via elevating miR-16 expression and decreasing protein levels of MMP-9 [160]. Table 4 provides the comprehensive summary of compiled data depicting the targets of osthole in several cancers.

Table 4. The anticancer potential of osthole along with its molecular targets.

Types of cancer	Cell lines	EC_{50}/ Conc.	Molecular Targets	References
Breast	4T1, MDA-MB-231, MDA-MB 453, MCF-7,	10, 15, 20, 40, 60, 80, and 100 μM	TβRII↓, p-Akt⊥, p-mTOR⊥, TGF-β-induced p-Smad2, p-Smad3⊥, c-Met↓, ERK↓, MMP-2⊥, Smad2↓, Smad3↓, Smad4↓, Smad7↓, NF-κB↓, RANK↓, TGF-β1↓	[39, 54, 59, 69, 80, 184, 190, 191]
Ovarian	A2780, OV2008	-	MMP-2/-9⊥	[192]
Lung	NCI-H460, A549, CL1-5, H1299, SK-LU-1	50, 100, and 150 μM	Bcl-2↓, MMP-2/-9↓, NF-κB⊥, Cyclin B1↓, p-Cdc2↓, Bax↑, PI3K/Akt⊥, IκB-α degradation⊥	[41, 54, 78, 166, 193]
Hepatocellular	HepG2, Hepa1-6, SMMC-7721	41, 82, 123, 164, and 205 μM	NF-κB↓, cleaved caspase-3↑, TNF↑, Bcl-2↑, TRAF↑, CARD↑, CIDE↑, MMP-2/-9↓, E-cadherin↑, beta-catenin↑, N-cadherin↓, vimentin↓	[54, 157, 158]
Leukemia	L1210	-	-	[154]
Prostate	PC3, DU145, LNCaP	-	-	[154]
Gastric	MK-1	-	-	[45]
Colon	SW480, SW620	15 μM	Cdc25 phosphatase↓, Cdc2/cyclin B↓, Bad↑, caspases-3/-7/-9^{Act}	[185]
Renal	ACHN, 786-O, Caki	-	E-cadherin↑, beta-catenin↑, vimentin↓, N-cadherin↓, Smad-3↓, Twist-1↓, Snail-1↓, MMP-2/-9↓, c-FLIP↓	[194, 195]
Cholangiocarcinoma	HCCC-9810, RBE	159 μM (48 h)	Bax↑, `Bcl-2↑, PARP↑,	[175]

		(HCC-9810) 153 μM (48 h) (RBE)	caspase-3, 9↑, p-Akt↓, PI3K↓	
Nasopharyngeal	NPC-039, NPC-BM	-	Bax↑, t-Bid↑, Bak↑, BimS↑, BimL↑, Fas↑, FADD↑, TNF-R1↑, RIP↑, TNF-R2↑, DcR2↑, p-ERK1/2↑, p-JNK1/2↑	[161]
Esophageal	KYSE150, KYSE410	198.45 μM (24 h), 102.51 μM (48 h) (KYSE150) 235.67 μM (24 h) 114.02 μM (48 h) (KYSE410)	Bcl-2↓, Cdc2↓, Cyclin B1↓, PARP1↓, Bax↑, cleaved PARP1↑, caspase-3, 9↑, p-AKT↓, PI3K↓, Survivin↓	[162]

↑Up-regulation; ↓Down-regulation; ⊥Inhibition; MMP-2: Matrix metalloproteinase-2; HGF: Hepatocyte growth factor; ; TGF-β1: Transforming growth factor-β1; FASN: Fatty acid synthase; cellular FLICE-like inhibitory protein (c-FLIP)

3.5. Osthole and its other biological activities

Various *in vitro* studies have given evidences that osthole has ability to promote osteoblast differentiation and proliferation [70, 196-198], to abolish osteoclast formation and to inhibit bone resorption [82, 199], these properties support osthole as one of the promising candidates to cure osteoporosis. Total coumarins of *Fructus cnidii* caused effective inhibition of bone turnover and conversed the process of bone loss at earlier stage of menopause in ovariectomized rats [200]. Osthole supports osteoblast differentiation by Wnt/β-catenin activation with subsequent increase of Bmp2 expression [201].

Osthole has also shown protective effects against hepatitis by suppressing hepatitis B virus (HBV) surface antigens secretion in HuH-7 and MS-G2 cells via increasing glycosylation of HBsAg [202]. It has ability to prevent hepatitis induced by concanavalin A in the *in vivo* models [36]. Osthole has also been proved to be protective against hyperlipidemic condition [55] as well as in alcoholic fatty liver of animals [72].

Osthole has a potential to accelerate beta-oxidation of hepatic fatty acids, thus, contributing towards repression of lipid accumulation in liver suggesting that it could be effective to prevent atherosclerosis [33]. Osthole, a bioactive natural entity, ameliorated fatty liver by reducing mRNA expression of FAS and LDL receptor in mice [55], mitigated insulin resistance via enhancing the release of adiponectin through activating (PPARα/γ) signaling cascade [203]. Osthole has known to possess anti-fibrotic effects in HSC-T6 hepatic cells [204]. It has also been

proposed as preventive agent against isoprenaline-induced fibrosis as it up-regulates the expression of PARα/γ with cessation of NF-κB signaling cascade in myocardial cells [52].

Additionally, osthole also possesses anti-diabetic properties. Various studies (*in vitro* and *in vivo*) investigations have revealed that osthole could mitigate hyperglycemia by activating PPARα/γ, and AMPK pathways [64, 205]. Osthole serves as vasorelaxant agent because of its antagonist effects on Ca^{2+} channel via up-regulation of cyclic guanosine monophosphate (cGMP) levels in vascular smooth muscles [34]. It also exhibits relaxing effects on blood vessels as well as on other cells and tissues such as taeniae coli, isolated guinea-pig ileum and trachea [100, 206], and on rabbit corpus cavernosum suggesting its effectiveness as relaxant agent [51]. Osthole is an anti-thrombotic agent that has potential to repress platelet coagulation by means of inhibiting thromboxane formation and breakdown of phosphoinositide [29, 207].

4. Conclusions and future perspectives

Natural products are torch bearers of specific advantages over traditional treatments being multi-targeted and less toxic, thus, providing a better version of remedy. Various naturally existing compounds have been declared as promising agents to suppress different cancer types in pre-clinical as well as clinical experimentation. Several reports have explored the role of osthole, one of bioactive constituent from variety of medicinal plants, as protective agent in several pathological conditions. Osthole has been known to inhibit cancer development and progression via modulating different signaling pathways. Although reported data acclaims that osthole possesses protective potential in various diseased conditions, however, further experimentation is required to evaluate pharmacokinetic profiles and molecular mechanism of osthole in depth as natural agent. In this way, a precise understanding of osthole can lead to better translation of its capabilities as anticancer agent in either pre-clinical or clinical perspectives in future.

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Conflict of interest

None

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