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Review

Recent advances in the therapeutic potential of emodin for human health

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ABSTRACT

Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is a bioactive compound, a natural anthraquinone aglycone, present mainly in herbaceous species of the families Fabaceae, Polygonaceae and Rhamnaceae, with a physiological role in protection against abiotic stress in vegetative tissues. Emodin is mainly used in traditional Chinese medicine to treat sore throats, carbuncles, sores, blood stasis, and damp-heat jaundice. Pharmacological research in the last decade has revealed other potential therapeutic applications such as anticancer, neuroprotective, antidiabetic, antioxidant and anti-inflammatory.

The present study aimed to summarize recent studies on bioavailability, preclinical pharmacological effects with evidence of molecular mechanisms, clinical trials and clinical pitfalls, respectively the therapeutic limitations of emodin. For this purpose, extensive searches were performed using the PubMed/Medline, Scopus, Google scholar, TRIP database, Springer link, Wiley and SciFinder databases as a search engines. The *in vitro* and *in vivo* studies included in this updated review highlighted the signaling pathways and molecular mechanisms of emodin. Because its bioavailability is low, there are limitations in clinical therapeutic use. In conclusion, for an increase in pharmacotherapeutic efficacy, future studies with carrier molecules to the target, thus opening up new therapeutic perspectives.

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1. Introduction

Natural products have played an important role worldwide in the treatment and prevention of human diseases [1–3]. Plants used for natural medicines come from various sources, including land plants, land microorganisms, and marine organisms, and their importance in modern medicine has been discussed in various reviews and scientific reports over time [4,5]. Medicinal plants are sources of new drugs and many modern drugs are produced indirectly from plants [6–8].

Emodin is the main active constituent of some herbs used in traditional Chinese medicine, such as *Rheum palmatum* L., *Reynoutria japonica* Houtt. (syn. *Polygonum cuspidatum* Siebold & Zucc), *Reynoutria multiflora* (Thunb.) Moldenke (syn. *Polygonum multiflorum* Thunb.) and *Senna obtusifolia* (L.) H.S.Irwin & Barneby (syn. *Cassia obtusifolia* L.) [9–12]. Emodin is a natural anthraquinone aglycone, also known as 1,3,8-trihydroxy-6-methylanthraquinone, and consists of an anthracene ring (tricyclic aromatic) with two ketone groups found in C9 and C10 (Fig. 1).

Anthraquinones are a group of polyphenolic secondary metabolites, most of which are produced by fungi, including *Penicillium* and *Aspergillus* species, and lichens, but also by higher plants and even some insects. In higher plants, anthraquinones are widespread and can be found as aglycones or as glycosylated forms[13]. Other common natural anthraquinone aglycones are rhein, aloe-emodin, chrysophanol and physcion, these latter two are derived from emodin [14].

Emodin was first isolated in 1925 and reported as ‘frangula-. Since then, it has been found in 94 plant species from many different families, although the main sources of emodin are plants of the *Fabaceae*, *Polygonaceae* and *Rhamnaceae* families [15]. Emodin is produced in both vegetative tissues and reproductive organs. In vegetative tissues, emodin has a role in protection against abiotic stresses, such as high light intensities, as a deterrent against herbivores allelopathic molecule against plant competitors, and antimicrobial agent against plant pathogens[15]. A high concentration of emodin in unripe fruits is important as a protection mechanism against insects and pathogens as well as a feeding deterrent against frugivores, to avoid premature food consumption [15]. In addition, residual emodin in ripe fruits appears to limit seed germination within the fruit and fruit consumption, thus favouring seed dispersion to new areas by multiple frugivores [16]. In traditional medicine, emodin has been used primarily as a laxative and for obesity-related diseases [17]. In addition to the main traditional uses, numerous studies have appeared reporting antitumoral properties of emodin [18,19]. The antineoplastic activities of emodin are based on its anti-mutagenic, anti-proliferative, anti-angiogenic and anti-metastatic activity as well as on the promotion of apoptosis and reversion of multidrug resistance [16,19,20]. However, many more pharmacological activities have been also reported in recent years. For example, emodin has been reported to have anti-inflammatory, anti-allergic, immunosuppressive, antioxidant, antifungal, antibacterial, antiviral, antiaging, antiosteoporotic, antidiabetic, cardioprotective, neuroprotective, hepatoprotective, nephroprotective activities [16,21]. This review summarized preclinical biological activities, clinical trials and therapeutic limitations of emodin.

2. Review methodology

The current paper reviews the available recent advances in the therapeutic potential, the molecular mechanisms of action and the signaling pathways of emodin. The PubMed/Medline, Scopus, Google scholar, TRIP database, Springer link, Wiley and SciFinder databases were chosen for the collection of articles published in English using the next MeSH terms: ”Emodin/metabolism”, “Emodin/pharmacology”, “Humans”, “Models, Biological”, “Mutagens/metabolism”, “Mutagens/pharmacology”, “Oxidation-Reduction”, “Animals”, “Apoptosis”. *In vitro* and *in vivo* pharmacological studies were screened and subsequent studies from the year 2014 to January 2021 have been collected.

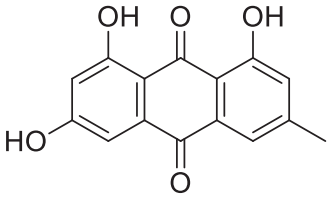

Fig. 1. Chemical structure of emodin.

Inclusion criteria: preclinical experimental studies were included that analyzed data on the bioavailability, pharmacokinetics of emodin, and molecular mechanisms of action, regarding the most important antioxidant, anti-inflammatory, neuroprotective and anticancer/chemoprotective effects.

Exclusion criteria: duplicate works, abstracts and conference data, experimental studies that included emodin associated with other drugs or homeopathic preparations.

The scientific names of the plant species were verified according to PlantList and the chemical formula with Chemspider [22,23].

3. Bioavailability and pharmacokinetics of emodin

3.1. In vivo studies

One of the earliest studies on this topic [24] evaluated the biological fate of emodin after intravenous (5.1 mg/kg) and oral (20–40 mg/kg) administration to experimental rats. It was observed that when emodin was given intravenously, the parent form of emodin diminished rapidly from the blood, whereas emodin glucuronides, ω-hydroxyemodin and ω-hydroxyemodin sulfates/glucuronides were produced. After oral administration of emodin, only glucuronide-derivates were detected in serum, whereas emodin, ω-hydroxyemodin and ω-hydroxyemodin sulfates/glucuronides were not found. These results indicated that first-pass metabolism may be the cause of the reduced bioavailability of emodin (< 3 %). Nevertheless, the serum metabolites of orally administered emodin showed higher free radical scavenging capacity compared to intravenous-administered parental compounds [24].

In a study carried out by Liu et al. [25], gender-dependent pharmacokinetics of emodin were evaluated in rats. After a single oral dose of emodin (8 mg/kg), plasma concentrations of total emodin in male rats were found to be 4-fold higher compared to female rats, which suggests a significant gender-dependent difference. This observation derives from a higher intestinal emodin absorption and lower intestinal excretion of the glucuronide form in male rats compared to female rats. However, after intravenous administration of emodin (4 mg/kg), plasma concentrations of emodin and emodin glucuronide showed no differences between genders. Plasma concentrations of emodin aglycone lowered biexponentially and were only detectable within 4 h in both the male and the female rats. In addition, emodin glucuronide was detected in plasma only 5 min after the intravenous administration in both genders. Overall, the authors concluded that oral bioavailability of emodin was reduced in rats (5 %) [25]. Lin et al. [26] investigated the dose-dependent pharmacokinetics of emodin after intragastrical (2 and 4 g/kg) and oral (4 g/kg) administration of *Reynoutria japonica* extract in rats. Brain, liver, lung, kidney and heart tissues were collected after oral administration of 7 doses of *R. japonica*. The authors reported that the free form of emodin was only detected in the liver, whereas emodin sulfates/glucuronides were mostly found in plasma, kidneys and lungs, but not detectable in the heart and brain [26].

Recently, some studies have been conducted to increase the low bioavailability of emodin. Accordingly, in a study carried out by [27], the influence of stilbene glucoside fraction obtained from *Radix Polygoni Multiflori* on the pharmacokinetics of the emodin fraction and its glucuronide was investigated. Emodin was orally administered to rats (82.4 mg/kg) after one week of intragastrical administration of stilbene glucosides (117 mg/kg). The results showed that pre-treatment with

stilbene glucosides, significantly increased the plasma concentrations of emodin, whereas those of emodin glucuronide were drastically reduced. The mechanism behind this observation was explained by the inhibition of the expression of UDP-glucuronosyltransferases (UGTs), particularly by the down-regulation of UGT1A8 mRNA expression [27]. Di et al. [28] investigated the pharmacokinetics of emodin in rats, alone and with co-administration of piperine (20 mg/kg each), to evaluate whether piperine could improve the oral bioavailability of emodin by inhibiting its glucuronidation. The results revealed that piperine increased the plasma emodin concentration and decreased the concentration of emodin glucuronide, hence, indicating a marked inhibition of UGTs [29]. Shi et al. studied [30], the distribution and pharmacokinetics of emodin suspension and emodin-loaded nanoemulsion (10 mg/kg) in plasma and tissues including liver, spleen, brain, heart, lung and kidney after oral administration to rats. The emodin-loaded nanoemulsion has been shown to significantly decrease the clearance rate of emodin concerning emodin suspension. Furthermore, pharmacokinetic analysis evidenced that emodin was mainly distributed in the liver, followed by the lung, kidney, heart or spleen, and the brain with the slightest concentration. Particularly, the mean residence time of emodin in the brain was nearly two-fold longer than that in the other tissues, indicating that emodin could have a high affinity for brain tissue [30]. In addition to the above, recently cocrystals of emodin with berberine chloride were prepared and characterized after being administered to rats by oral gavage (40 mg/kg). The obtained results highlighted that the relative bioavailability of cocrystals was 1.2–1.7 times that of pure emodin [31]. Some recent *in vivo* animal studies on the bioavailability of emodin are presented in Table 1.

3.2. *In vitro studies*

As *in vivo* studies, a few *in vitro* researches have also been conducted to increase the bioavailability of emodin. Most of the *in vitro* research on the bioavailability of emodin is mechanistic studies (Table 2).

One of the first reports on this matter [32] investigated the intestinal absorption characteristics of emodin by determining the intracellular accumulation across intestinal Caco-2 cells. The authors observed that inhibitors of intestinal transport, verapamil and cyclosporine, favored the absorption of emodin, whereas phloridzin inhibited it, all in a dose-dependent manner. The authors suggested that P-glycoprotein or multidrug-resistant protein (MRP), localized in the apical or basolateral membrane, was capable of effluxing emodin, effectively opposing absorption and intracellular accumulation. In addition, it has been proposed that intestinal absorption of emodin could be transported by sodium-glucose cotransporters (SGLT1) [32]. In a study conducted by Song et al. [33], emodin was incubated with rat liver microsomes in the presence of an NADPH-generating system to investigate the metabolism of the compound *in vitro*. The parental compound and six metabolites – ω-hydroxyemodin, 2-hydroxyemodin, 3-carbomethoxy-6-methoxy-1, 8-dihydroxyanthraquinone, 4-hydroxyemodin, emodic acid, and physcion - were detected after incubation with microsomes [33].

In a more comprehensive study [34], liver microsomes from several species were used along with a rat intestinal perfusion model and rat intestinal microsomes to study the effect of species and gender differences on emodin metabolism. Emodin glucuronidation in liver microsomes was species-dependent and varied between males and females. It was also found that the excretion rates of emodin-3-*O*-glucuronide in the rat intestine, were different depending on the region of the intestine (duodenum, upper jejunum, terminal ileum, and colon). The glucuronidation rates were substantially quicker in male mice than in females probably derived from an increased expression level of UGT2B1 (2-fold) in the male liver. Emodin was considered to be rapidly glucuronidated as the intrinsic clearance values were higher than that of isoflavones. Although the rapid metabolization by UGTs is proposed as the main reason for emodin's poor bioavailability, another reason could also be related to very poor solubility (< 1 μM).

Table 1
In vivo studies regarding the bioavailability of emodin.

Species	Dose	Design	Results	Ref.
Rats	5.0 mg/kg, iv 20–40 mg/kg, orally	blood sampling	intravenous: ↓reduction, ↑emodin glucuronides orally: ↑emodin glucuronides	[24]
Rats	8 mg/kg, single dose, orally 4 mg/kg, single dose, iv	blood sampling	orally: ↑plasma concentrations intravenously: no difference in the plasma concentrations	[25]
Rats	intragastrically: 2 and 4 g *Reynoutria japonica* (pc)/kg 4 g pc/kg, seven doses orally	blood sampling brain, liver, lung, heart, kidney sampling	orally: emodin sulfates/ glucuronides were found in organs	[26]
Rats	82.4 mg/kg, single dose orally	blood sampling	↑plasma concentrations	[27]
Rats	20 mg/kg, single dose, orally	blood sampling	co-administration of piperine (20 mg/kg) effects: ↑emodin concentration in plasma ↓emodin glucuronide	[29]
Rats	10 mg/kg, single dose, orally	blood sampling brain, heart, spleen, kidney, liver, lung sampling	emodin-loaded nanoemulsion: ↓ the clearance rate of emodin	[30]
Rats	40 mg/kg, single dose intragastrically	Blood sampling	↑bioavailability of cocrystals with emodin	[31]

In a study carried out by the same research group [35], the absorption and excretion of emodin and its metabolites were further examined using intestinal CaCo-2/TC7 cells. The study showed that passive diffusion is the main mechanism of emodin transport in the Caco-2 cell monolayers, and phase II conjugates are excreted or/and absorbed by the intestinal cells by the action of the multi-drug resistance protein (MRP) transporters [35]. An emodin nanoemulsion containing a chromophore was assessed for its capability to enhance transcellular absorption of emodin using UGT1A1-overexpressing MDCKII cells (MDCK1A1 cells) [36]. The results revealed a marked reduction in total emodin glucuronidation in the emodin nanoemulsion group compared to the control group. The mechanism behind this observation was suggested to be associated with the inhibition of UGT1A1-mediated glucuronidation of emodin by cremophor EL [36]. Qiong et al. [37] evaluated the effect of 2, 3, 5, and 4′-tetrahydroxystilbene-2-*O*-β-D-glucopyranoside (TSG) on the cellular absorption and microsome metabolism of emodin in the human liver. The results evidenced that after TSG treatment, the absorption of emodin in Caco-2 cells was improved, whereas the metabolism of emodin was inhibited in liver microsomes [37].

4. Preclinical studies of emodin: potential mechanisms behind pharmacological activities

This section summarizes the comprehensive information on the main biological activities of emodin including antioxidant, anti-inflammatory, neuroprotective and anticancer (Fig. 2). The main mechanisms of action of emodin have been summarized in Table 3 for the anticancer effects and Table 4 for the other therapeutic effects.

Table 2
In vitro studies regarding the bioavailability of emodin.

Cell type/tissue	Dose	Design	Results	Ref.
Caco-2 cells	2.5–200 μm	2 h incubation at 37 °C and 4 °C	↑absorption by verapamil and cyclosporine ↓absorption by phloridzin	[32]
Rat liver microsomes	100 μm	60 min incubation at 37 °C	metabolism in the liver	[33]
Mice, rats, dogs, guinea pigs human liver microsomes; Rat intestinal perfusion model; Rat intestinal microsomes	2.5–40 μm	10 min incubation at 37 °C	↑excretion rates of emodin-3-*o*-glucuronide in rats'intestine	[34]
Caco-2/TC7 cells	2.4–13 μm	2 h incubation at 37 °C	mechanism of emodin transport: passive diffusion phase ii conjugates are excreted/absorbed by the intestinal cells *via* the action of the MRP transporters	[35]
Liver and intestinal microsomes from rats and humans	0.5 and 5 μm	10 min incubation at 37 °C	glucuronides were formed by multiple human UGT isoforms with 1a9 being the most prominent	[38]
MDCKII cells	10 μm	2 h incubation at 37 °C	↓glucuronidation in the emodin nanoemulsion group compared to the control group	[36]
Caco-2 cells human liver microsomes	50 μM	incubation at 37 °C for up to 2 h	2, 3, 5, 4′-tetrahydroxystilbene-2-o-β-D-glucopyranoside: ↑ absorption in intestinal cells ↓metabolism in human liver microsomes	[37]

Symbols:↑ increase, ↓decrease.

4.1. Antioxidant

Oxidation is a common and essential process for the sustainability of biochemical reactions [69,70]. However, disruption in the oxidation control mechanism may lead to the excessive production of reactive oxygen species (ROS) [5,71,72]. When the intrinsic antioxidant defense mechanisms can not neutralize the generated ROS, the imbalance between ROS and antioxidants can instaurate a situation of oxidative stress in the body [73,74]. In this case, ROS interfere with the activity of a wide array of biomolecules such as proteins, lipids and DNA resulting in oxidative damage which is directly associated with diverse pathological conditions including inflammatory disorders, diabetes, cardiovascular diseases, and also cancer [75,76].

4.1.1. In vitro studies

Cisplatin is an anticancer agent commonly utilized for the treatment of several cancer types including breast, ovarian, bladder, and pancreatic cancers. However, the long-term use of cisplatin leads to drug accumulation within the mitochondria and altered mitochondrial DNA and protein damage by the stimulation of oxidative stress [77]. In a study by Yan et al. [78] emodin treatment (5 μM) was capable to reduce the intracellular ROS production induced by 0.1 μM cisplatin in human osteosarcoma MG-63 cells. Also, cisplatin-induced translocation of the nuclear factor erythroid 2 related factors 2 (Nrf2) was induced with cisplatin treatment while its translocation was partially suppressed by emodin. In addition, emodin decreased the cisplatin-induced Nrf2/antioxidant response element (ARE) signaling pathway evidenced by using ARE-luciferase reporter plasmid.

In the study by Chen et al. [79], the beneficial effects of emodin against oxidative stress have been investigated in HK-2 human renal tubular cells subjected to sequential episodes of hypoxia and reoxygenation (H/R) to induce oxidative stress. Then, cells were maintained in normoxic conditions (5 % CO_2, 21 % O_2 and 74 % N_2) for 12 h. H/R exposure led to a significant reduction in cell viability, which was prevented in a dose-dependent manner by emodin pre-treatment (10, 30, and 50 μM). Moreover, H/R caused an increase in the generation of ROS and malondialdehyde (MDA) together with a substantial reduction in the enzymatic activities of the antioxidant defense system including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GP_X) compared to the control group. Pre-treatment with emodin significantly decreased the ROS and MDA production induced by H/R and recovered the activities of the antioxidant enzymes in a concentration-dependent manner [79].

Zhao et al. [80] reported the antioxidant effects of emodin in peripheral blood leukocytes isolated from the Wuchang bream, *Megalobrama amblycephala.* Isolated leukocytes were introduced to 96-well plates and exposed to several concentrations (0.04, 0.20, 1, 5, 25 μg/mL) of emodin. The results showed that low concentrations of emodin (0.04 and 0.20 μg/mL) significantly increased the gene expression of the antioxidant *Cu-Zn* SOD, whereas at 1 μg/mL also significantly up-regulated the mRNA expression of CAT, NADPH oxidase 2 (NOX2), and nuclear factor erythroid 2-related factor 2 (Nrf2) genes. At these concentrations, emodin improved antioxidant defense through transcriptional regulation levels of antioxidant enzymes [80]. However, at higher concentrations, emodin significantly increased ROS generation and reduced cell viability.

In an *in vitro* assay, HepG2 cells were treated with arachidonic acid and iron to induce oxidative stress. In these cells, the pre-treatment with emodin (3, 10, 30 μM) for 1 h increased cell viability and the levels of cleaved caspase-3 and, ameliorated the elevated levels of ROS induced by the treatment with arachidonic acid + iron. The mechanistic analysis confirmed that emodin treatment activated the liver kinase B1 (LKB1) 1-AMPK pathway and upregulated the phosphorylation of yes-associated protein 1 (YAP1) and large tumor suppressor kinase 1 (LATS1), downstream targets of the Hippo signaling pathway.

4.1.2. In vivo studies

Regarding animal studies, Yao et al. [81] evidenced the potential antioxidant effect of emodin (1 mg/kg) administered once in a Sprague Dawley rat model of severe acute pancreatitis (SAP) induced by administration of sodium taurocholate into the biliary-pancreatic duct. At regular intervals (1, 3, 6, and 12 h), blood samples and pancreatic tissues were collected for analysis. In the pancreatitis-induced group, the levels of MDA in serum and pancreatic tissue were significantly increased for each incubation period while emodin treatment was able to effectively suppress this increase. On the contrary, SOD levels were decreased in the pancreatic tissues and blood obtained from the pancreatitis group and this increase was partially recovered by emodin.

A study using the same animal model also investigated the protective effects of three-time intragastric administration of 6 mg/mL emodin (1 mL/100 g) [82]. Emodin administration significantly reduced histological alterations in the pancreas induced by sodium taurocholate and reduced the circulating levels of lipase, amylase, TNF-α, and IL-18. Moreover, emodin modulated inflammasome-related mediators evidenced by a reduction in NOD-like receptor protein 3 (NLRP3) and an increase in voltage-dependent anion channel 1 (VDAC1), a mitochondrial porin. In isolated neutrophils, emodin was capable to reduce the production of ROS. The authors concluded that emodin can improve the symptoms of SAP rats with modulation of ROS production and inflammasome pathway.

Nemmar et al. [83] investigated the antioxidant effect of emodin in the lungs of BALB/C mice intratracheally exposed to 20 μg of diesel

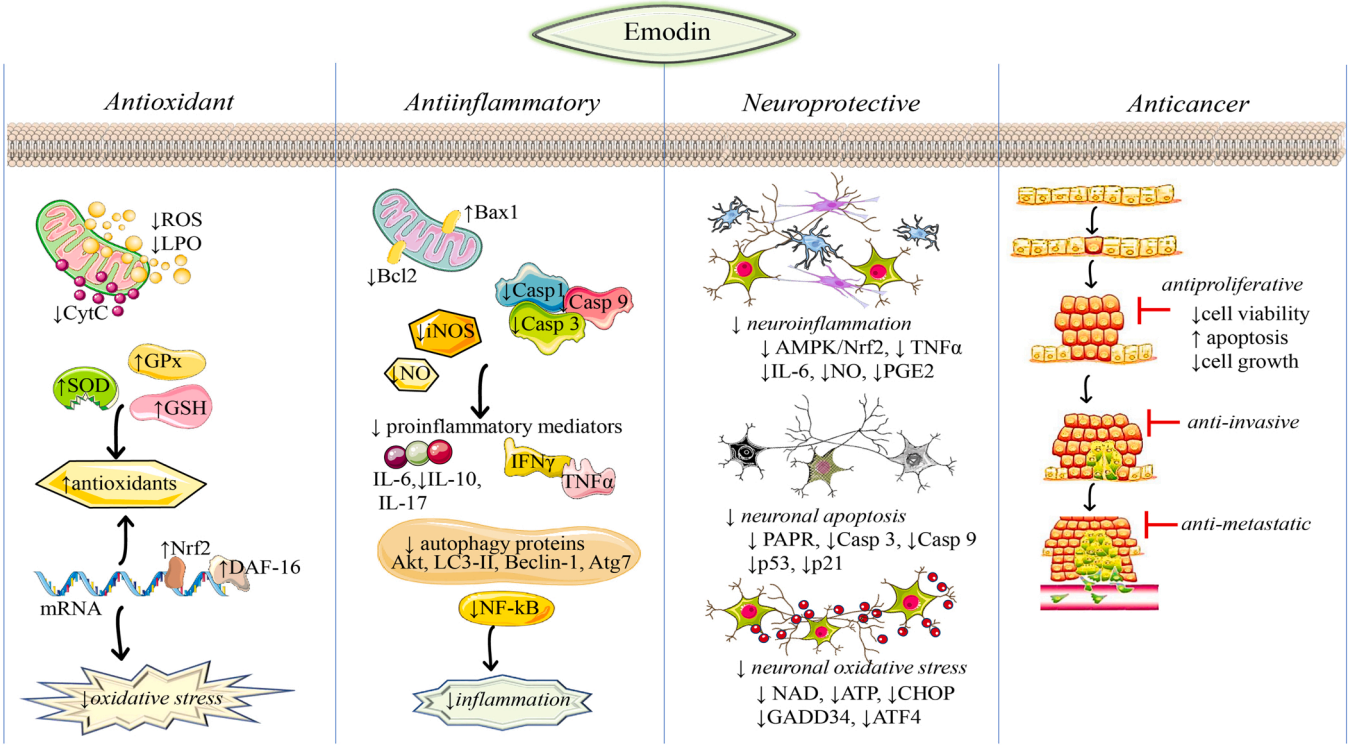

Fig. 2. Diagram with the most representative pharmacological effects of emodin and molecular mechanisms of action. Abbreviations and symbols: ↑ increase, ↓decrease, ROS: Reactive oxygen species, LPO: lipid peroxidation, SOD: superoxide dismutase, NO: nitric oxide, GPx: glutathione peroxidase, NRF: 2nuclear factor erythroid 2–related factor 2, DAE-16 abnormal DAuer Formation-16, Casp: caspase, IFNγ: interferon γ, IL: interleukin, AMPK: AMP-activated protein kinase, TNF: tumor necrosis α, NF-κB: nuclear factor kappa B, PGE2: prostaglandin E2, GADD 34: growth arrest and DNA damage-inducible gene 34, ATF4:activating transcription factor 4.

Table 3
Anticancer effects and mechanism of action of emodin.

Effect	Cell lines tested/ *in vitro*	Proposed mechanisms of action	Ref.
Apoptosis induction	bladder cancer cell	↑epigenetic modification ↓ cancer cells growth	[39]
	A549 cells	↑apoptosis ↓cancer cells growth	[40]
	MCF-7 cells	↑ apoptosis ↑apoptosis-related genes	[41]
	glioma stem cells	↓b-catenin, ↓Notch-1, ↓STAT3	[42]
	neuroblastoma cells	↓ cancer cells growth	[43]
	Ewing's sarcoma cells	↓EGFR/EGFRvIII,	
	T-cell leukemia cells	↑ Hsp90	
	colon adenocarcinoma cells		
	hepatocellular carcinoma cells	↑TNF-α, ↑apoptosis ↑ cancer cells death	[44]
	breast cancer cells	↓ cancer cells growth, ↓ERα	[45]
Cell cycle arrest	SMMC-7721 cells	↓proliferation	[41]
Anti-metastasis	breast cancer cells	↓ pulmonary metastasis ↓STAT6 phosphorylation ↓CCAAT/enhancer-binding protein β	[46]
	breast cancer cell	emodin combined with curcumin ↓proliferation, ↑miR-34a	[41]

Abbreviations and symbols: ↑ (increase),↓(decreased), signal transducer and activator of transcription 3 (STAT3), signal transducer and activator of transcription 6 (STAT6), estrogen receptors alpha (ERα), microRNA-34a (miR-34a).

exhaust particles (DEP). DEP exposure significantly increased the degree of lipid peroxidation (LPO) and ROS production whereas reduced glutathione (GSH) concentration in the control group. The treatment with emodin 4 mg/kg significantly recovered all these biomarkers 24 h after DEP exposure. In terms of safety concerns, no apparent toxic effects were observed in the model animals associated with emodin treatment [83]. The same research group investigated the protective effects of emodin on the heart tissue [84] Using the same experimental procedure as in the previous study, DEP exposure led to a reduction in the levels of SOD, which was reversed by emodin treatment. Interestingly, DEP exposure caused an increase in the antioxidant glutathione reductase (GR), whereas the treatment with emodin normalized the enzyme levels [84]. The study of Xue et al. [85], evaluated the pro-oxidant activity of exposure to cigarette smoke in the lungs of C57BL/6 mice. One hour before each cigarette smoke exposure during four experimental days, mice were pretreated with emodin (20 or 40 mg/kg) or vehicle (0.5 % DMSO).

Cigarette smoke significantly decreased the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GP_X) [86]. Emodin treatment ameliorated the decreased activities of these enzymes in a dose-dependent manner. Moreover, emodin treatment resulted in increased enzymatic activity and upregulated the gene expression of heme oxygenase-1 (HO-1) and protein levels for the control group. Additionally, emodin treatment induced a significant increase in the mRNA and protein levels of Nrf2.

The ageing process is highly correlated with exposure to oxidative stress throughout life [72,87,88]. In the study by Zhao et al. [89], the antioxidant capacity of emodin was investigated in the worms (*Caenorhabditis elegans*) as a model organism. 150 μM of emodin treatment provided a significant reduction in ROS production and a positive effect on the lifespan of worms under both paraquat and heat shock stress conditions. Additionally, quantitative RT-PCR analysis demonstrated that emodin up-regulated the expression of the transcription factor DAF-16 which is associated with ageing and longevity, as well as some of its downstream antioxidant and anti-thermal enzymes including SOD,

Table 4
Other biological effects and mechanisms of action of emodin.

Biological activities	Type of study	Mechanisms of action/ Results	Ref.
Anti-inflammatory	C2C12 myoblast cells *in vitro*	↓apoptosis, ↑ Bax /Bcl-2 ratio, ↑ROS, ↓caspase-3 ↓PARP, ↓LC3-II, ↓Beclin-1, ↓Atg7, ↓Akt	[47]
	sensitized with ovalbumin C57BL/6 mice *in vivo*	↓ IL-4, ↓IL-5, ↓IL-6, ↓MMP-9, ↓IgE ↓NF-κB, ↓MAPK, ↓PI3K/ Akt, ↓NIK-IKK	[48]
	model of collagen-induced arthritis mice *in vivo*	anti-arthritis effects ↓NF-κB, ↓pro-inflammatory mediators: ↓IL-6, ↓TNF-α, ↓NO	[49]
	ConA-stimulated mouse splenocytes *in vitro*	↓splenocyte proliferation ↓progression of cells to the S phase ↓proinflammatory cytokines: TNF-α, IFNγ, IL-6, IL-17, IL-2 ↓anti-inflammatory cytokines: IL-4, IL-10	Sharma et al. [50]
	LPS-stimulated RAW264.7 macrophage cells *in vitro*	↓NF-κB, ↓PPAR-γ, ↓mRNA, siRNA-PPARγ ↓ICAM-1, ↓MCP-1, ↓TNF-α	Zhu et al. [51]
	hypoxia-exposed rat cardiomyocytes H9c2 *in vitro*	↓p53, ↓p21, cyclin D1 ↓caspases-3, -9, ↓miR-138 ↓Sirt1/Akt, ↓Wnt/b-catenin	Zhang et al. [52]
Antiviral	MDCK cells Influenza A H7N9 virus *in vitro*	↓replication of influenza A ↓antiviral genes ↓ IFN-β, ↓IFN-γ, ↓2′5’,-OAS	[53]
	Vero E6 cells Retrovirus *in vitro*	blocks the S protein, blocks ACE2 interactions ↓ infectivity of S protein-pseudotyped	[54]
Antibacterial	Gram-positive bacteria *Bacillus subtilis, Staphylococcus aureus in vitro*	bacteriostatic effect	[55]
	Gram-negative bacteria *Klebsiella pneumonia, Escherichia coli*) *in vitro*	no effect	[55]
Anti-allergic	hapten-induced dermatitis in NC/Nga mice *in vivo* rat basophilic leukemia RBL-1 cells *in vitro*	↓mast cell activation, ↓anaphylactic reactions, ↓receptor-proximal Syk-dependent signaling pathways ↓LOX	[56]
Neuroprotective	LPS-stimulated mouse primary microglia BV-2 microglial cells *in vitro*	↑ Bcl-2, ↓ amyloid-β25–35-induced autophagy, ↑ER/PI3K/ Akt ↓LKB1, ↓CaMKII, ↓Nrf-2, NQO1, ↓TNF-α,↓↓ IL-6, ↓NO, ↓PGE2	[57]
	human neuronal SH-SY5Y cells *in vitro*	↓ OGD/R, ↓PARP, ↓caspase-3, ↓caspase-9, ↓p53, ↓p21, ↓ Bax, ↑expression of antioxidant genes:↑ NQO1, ↑HO-1 ↑Nrf-2/ARE, ↑AMPK, ↑GSK3β	[58]
	model of vascular dementia and Parkinson’s disease SH-SY5Y cells *in vitro*	↑cell viability, ↓apoptotic cell ratio ↓LDH, ↓MT1, ↓MT2, ↓ZnT-1 ↓intracellular levels of NAD^+ and ATP ↓ROS, ↓mRNA, ↓CHOP, ↓ADD34, ↓ATF4	Liu et al. [59]
	model of methylglyoxal-induced mitochondrial dysfunction SH-SY5Y cells *in vitro*	↓AMPK/Nrf2/HO-1	[60]
	model of chronic stress mice *in vivo*	↓progression of behavioral impairments in mice ↓consumption of sucrose, ↓plasmatic corticosterone, ↓mRNA, ↓BDNF, ↓GR, ↓protein expression levels in the hippocampus	[61]
	model of cerebral infarction SD rats *in vivo*	↓cerebral I/R-induced neurological deficit ↓Cx43, ↓AQP4 ↓ the progression of ischemic stroke ↓neuronal cell death, ↓cerebral edema	Li et al. [62]
	model of Aβ-induced autophagy double-transgenic mice *in vivo* PC12 cells *in vitro*	↓conversion of microtubule-associated LC3-I to LC3-II ↓cells autophagy, ↓Aβ25–35 ↑cell viability ↓LDH, ↑Bcl-2, ↓Beclin-1,↑ PI3K/Beclin-1/Bcl-2	Sun et al. [63]
	model of photothrombotic ischemia mice *in vivo* model of glutamate-induced oxidative toxicity HT22 mouse hippocampal neuronal cells *in vitro*	↓cerebral infarct volume ↑motor skill score ↓apoptosis and ↑viability of HT22 cells ↓LDH, ↑Bcl-2, ↑↑pAKT, ↑p-cyclic-AMP, ↑pCREB, ↓Bax, ↑caspase-3	Ahn et al. [64]
	model of I/R induced by right middle cerebral artery occlusion rats *in vivo* cells exposed to oxygen-glucose deprivation PC12 cells *in vitro*	↓infarct size ↑percentage of recovery in body asymmetry ↑cell viability ↑release of glutamate ↓ROS, ↑Bcl-2, ↑GLT-l, ↑caspase-3, ↑ERK1/2	Leung et al. [65]
Anti-osteoporotic	rats *in vivo*	↓ bone resorption	[66]
Anti-diabetic	3 T3-L1 adipocytes *in vitro*	↑PPARγ, ↑glucose metabolism	[66]
	insulin-resistant HepG2 cells *in vitro*	↑glucose uptake by cells	Jung et al. [136]
	diabetic rats *in vivo*	↓hyperglycemia, ↓α-glucosidase, ↓blood glucose level, ↓maltase activity, ↓α -glucosidase	Li et al. [67]
Hepatoprotective			[68]

(*continued on next page*)

Table 4 (*continued*)

Biological activities	Type of study	Mechanisms of action/ Results	Ref.
	rats *in vivo*	↓ethanol-mediated liver steatosis ↓ ALT, ↓AST, ↓ TGL	

Abbreviations and symbols: ↑increase, ↓ decrease, Angiotensin-converting enzyme 2 (ACE2), alanine aminotransferase (ALT), aspartate aminotransferase (AST), aspartate aminotransferase mitogen-activated protein kinase (MAPK), B-cell lymphoma protein 2 (Bcl-2), triglycerides (TGL), IκB kinase (IKK); NF-κB-inducing kinase (NIK), Nuclear factor-kappa B (NF-κB), mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)/AKT, response element-binding protein (pCREB), glutamate transporter-1 (GLT-l), serine-threonine liver kinase B1 (LKB1), serine-threonine liver kinase B1 (LKB1), quinone acceptor oxidoreductase 1 (NQO1), prostaglandin E2 (PGE2), lactate dehydrogenase (LDH), zinc transporter families (MT1, MT2, and ZnT-1) oxygen-glucose deprivation/reoxygenation (OGD/R), microtubule-associated protein light chain 3 (LC3) brain-derived neurotrophic factor (BDNF), glucocorticoid receptor (GR), connexin 43 (Cx43), aquaporin 4 (AQP4), phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2), interleukin (IL)-6, tumor necrosis factor (TNF)-α, nitric oxide (NO), concanavalin A (ConA), interferon γ (IFNγ), nuclear factor(NF)-κB, peroxisome proliferator-activated receptor (PPAR)-γ.

CAT, and heat shock protein (HSP)-16.2. These novel findings suggested that emodin could be an alternative anti-ageing agent through its antioxidant properties [89].

Tian et al. [90] evidenced the antioxidant function of emodin in a bleomycin-induced idiopathic pulmonary fibrosis rat model. After 10 days of bleomycin exposure, emodin (20 mg/kg) was orally administered to the animals once a day for 21 experimental days. Similar to the previous study, Emodin treatment effectively induced the translocation of Nrf2 into the nucleus along with a significant and potent rise in the expression level of the antioxidant HO-1. Moreover, the levels of antioxidants such as GSH, GPx and SOD were upregulated by emodin treatment when compared to the bleomycin-induced group. Bleomycin exposure led to higher MDA production as compared to the control group, while emodin decreased its production significantly [90].

In the study by Lee et al. [91] the antioxidant activity of emodin (10 and 30 mg/kg) was investigated in the liver of acetaminophen (paracetamol, APAP)-induced acute liver damage, mouse model. Pre-treatment with emodin significantly reduced histological liver damage induced by APAP, reduced the release of transaminases and recovered the protein levels of GPx. Next-generation sequencing (NGS) analysis evidenced that most of the induced genes were targets of the AMPK and the Hippo signaling pathways.

A recent study was designed to evaluate the effects of a nanosystem loading emodin (5 and 20 mg/kg/d for 5 days, orally administered) in a mouse model of acute colitis induced by dextran sulfate sodium [92]. The treatment with the nanosystem significantly improved the disease activity index and the histological alterations to a greater degree than free emodin. Also, the nanosystem was capable to reduce oxidative stress and inflammation by dropping the gene expression of inducible nitric oxide synthase (iNOS), TNF-α, and IL-1β, the protein levels of myeloperoxidase (MPO) and increasing the levels of GSH. In addition, loaded emodin significantly reduced the hepatotoxicity induced by free emodin. The authors concluded that the new formulation could be an alternative to available therapy for ulcerative colitis but with reduced side effects.

All these findings suggest that emodin could be a promising antioxidant agent for the prevention and treatment of various degenerative and inflammatory disorders.

4.2. Anti-inflammatory

Chronic inflammation is the main source of the progression of a wide spectrum of diseases such as type II diabetes, metabolic syndrome, hypoxia-reperfusion injury, neurodegeneration as well as cancer [93, 94]. Recent *in vitro* and *in vivo* studies confirmed that emodin can be a potential therapeutic agent against various inflammation-related diseases.

4.2.1. In vitro studies

Chen et al. [95] investigated the anti-inflammatory and anti-apoptotic effects of emodin in the TNF-α-induced myoblasts cells to understand the action mechanism of emodin in the model of muscle atrophy. In this study, TNF-α was utilized to induce muscle atrophy in C2C12 myoblast cells since the pro-inflammatory cytokine released at a high level in muscle stimulates apoptosis by mitochondrial pathway, ROS production and autophagy. TNF-α treatment with 20 ng/mL stimulated apoptosis in the myoblasts associated with an increased Bax /Bcl-2 ratio, ROS production, cleaved-caspase-3, and cleaved poly (ADP-ribose) polymerase (PARP) which were all downregulated after 24 h of emodin treatment. Moreover, emodin inhibited the loss of mitochondrial membrane potential (ΔΨm) in TNF-α-treated C2C12 myoblasts. In addition, emodin downregulated the expression of autophagy-related proteins such as LC3-II, Beclin-1, and Atg7 concerning the TNF-α induced group. In TNF-α treated myoblasts, the level of phosphorylated Akt form, which is related to apoptosis and autophagy modulation, was significantly decreased, and emodin treatment effectively recovered this level. The authors concluded that emodin suppresses the mitochondrial apoptosis pathway and autophagy *via* the activation of Akt in the myoblast cells challenged with the pathological level of TNF-α [95].

In a study conducted by Li et al., emodin (10, 20, 50, and 100 μM for 24 h) has been reported to protect neuroblastoma SH-SY5Y cells against H_2O_2-induced apoptosis and inflammation [96]. Emodin significantly improved cell viability, inhibited the expression of caspase-3 and -9 and reduced the release of lactate dehydrogenase and pro-inflammatory mediators - interleukin (IL)-6, tumor necrosis factor (TNF)-α and nitric oxide (NO) – induced by H_2O_2-exposure. In addition, emodin treatment ameliorated the mitochondrial disfunction reducing ROS production and cytochrome c release, recovering NAD^+ and ATP levels and protecting from membrane potential loss. The mechanism of action was associated with the inhibition of the PI3K/mTOR/glycogen synthase kinase-3 (GSK-3) signaling pathway.

Sharma et al. [50] designed a study to evaluate the mechanism associated with the immunomodulatory and anti-inflammatory effects of emodin on concanavalin A (ConA)-stimulated mouse splenocytes. Isolated splenocytes from naive male Swiss mice were exposed to 10 μM of ConA as a mitogen to stimulate T-lymphocyte proliferation which is encountered in the progression of many inflammatory diseases. The cytotoxic doses of emodin in the unstimulated splenocytes were greater than 100 μM, therefore the following experiments were performed with 100 μM of emodin. After 24 h incubation of ConA-treated splenocytes, cell proliferation was increased by 43.3 % in the control group. Emodin treatment (100 μM) for 18 h following the primary stimulation with ConA for 6 h significantly reduced splenocyte proliferation. Moreover, emodin did not exert a significant effect on the cellular cycle of unstimulated splenocytes which usually stay in the G1/G0 phase. ConA stimulation induced the progression of cells to the S phase, which was effectively decreased by emodin exposure. Emodin treatment of ConA stimulated splenocytes also resulted in a significant decrease in the production of proinflammatory cytokines such as TNF-α, interferon γ (IFNγ), IL-6, IL-17, and IL-2 as well as a remarkable increase in anti-inflammatory cytokines like IL-4 and IL-10 [50].

The anti-inflammatory effect of emodin through nuclear factor(NF)-κB and peroxisome proliferator-activated receptor (PPAR)-γ dependent pathways in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophage cells was suggested by Zhu et al. [51]. LPS treatment significantly downregulated the mRNA and protein levels of PPARγ, which was considerably reversed by emodin treatment. In addition, RAW264.7

cells were transfected with siRNA-PPARγ to knock down PPARγ expression to determine the participation of this transcription factor in the regulation of inflammatory mediators and the protective effects of emodin in LPS-stimulated cells. According to the results, while emodin dose-dependently decreased the expression of ICAM-1 and MCP-1 and the production of TNF-α at the concentrations of 1, 5 and 25 μM, these effects were significantly abolished by siRNA-PPARγ transfected cells. Similarly, emodin at 25 μM inhibited the LPS-induced activation and DNA binding activity of NF-κB p65 and, this effect almost disappeared in siRNA-PPARγ transfected RAW264.7 cells indicating that emodin exerts its anti-inflammatory effects through a PPARγ-dependent pathway [51].

An *in vitro* study by Liang et al. [97] described the anti-inflammatory effect of emodin evidenced by a decrease in the percentage of apoptotic cells and inhibition of the Notch and NF-κB pathways in LPS-induced murine chondrogenic ATDC5 cells to mimic osteoarthritis disease. According to the cell viability assay, 10 mM the concentration of emodin was selected since it was non-toxic for non-treated cells. Moreover, the expression of Notch receptors (Notch1, Notch2, and Notch3) and phosphorylated levels of p65 and IκBα in the NF-κB pathway were remarkably reduced by cotreatment of emodin and LPS in ATDC5 cells compared to LPS-treated cells. Emodin regulation of the Notch and NF-κB gene expression was found to be strongly connected to the induction of taurine upregulated gene 1 (TUG1). This mechanism of emodin was analyzed with the knockdown of TUG1gene in ATDC5 cells that were transfected with short-hairpin RNA directed against human long non-coding RNA TUG1 (sh-TUG1) or non-targeting sequence (sh-NC). A reduced expression level of TUG1 was observed in the sh-TUG1 group compared to sh-NC and, the ameliorative effect of emodin on the Notch and NF-κB pathways was reversed in the sh-TUG1 transfected cells concerning the sh-NC transfected cells. These results might suggest that emodin can inhibit the Notch and NF-κB pathways *via* up-regulating TUG1 [97].

Zhang et al. [52] evidenced the protective effect of emodin in hypoxia-induced injury in rat cardiomyocyte H9c2 cells. The safe concentration of emodin on normal H9c2 cells was determined as 20 μM. In hypoxia-exposed H9c2 cells, 15 μM of emodin down-regulated both p53 and p21 expressions while the expression of cyclin D1 was up-regulated. Emodin also resulted in a lower ratio of cleaved-/pro-caspases-3 and -9 when compared to the control group reducing apoptosis and increasing cell viability. In addition, hypoxia exposure significantly reduced the expression of miR-138 which is essential for cardiac cell fate and its levels were recovered by emodin. The function of miR-138was investigated in miR-138 transfected H9c2 cells or using a specific inhibitor. The up-regulation of miR-138 in transfected cells ameliorated hypoxia-induced cell injury and potentiated the emodin effects whereas its inhibition reduced the protective effects of emodin on injured cells. The underlying mechanisms were associated with the activation of Sirt1/Akt and Wnt/b-catenin signaling pathways. MLK3 protein was negatively modulated by miR-138 expression in hypoxia-induced cells inhibiting these pathways. Therefore, emodin enhanced the decrease in the activation of Sirt1/Akt and Wnt/b-catenin signaling pathways in hypoxia-treated cells. In conclusion, emodin treatment might be an ameliorative alternative in hypoxia-induced injury *via* the down-regulation of MLK3 mediated by miR-138 [52]. In an *in vitro* assay, emodin treatment was capable to promote apoptosis in isolated PMNs stimulated with LPS. Consistently, emodin markedly increased the mRNA and protein expression of the proapoptotic Fas, FasL, Bax, cleaved caspase-3 and decreased the antiapoptotic Bcl-xL expression in PMNs. Some pathologic symptoms such as edema, hemorrhage, acinar cell necrosis, and inflammatory cell infiltration were observed in SAP-induced pancreatic tissue, however, these symptoms were relieved in the emodin-treated group. Additionally, emodin treatment diminished the pulmonary and pancreatic edema by decreasing the wet/dry ratio of lung and pancreatic tissue when compared to the SAP-induced group [98].

4.2.2. *In vivo studies*

Moving on to *in vivo* studies, Ma et al. [99] evidenced the beneficial effects of emodin (10 mg/kg i.p.) in a rat model of blast-induced traumatic brain injury (bTBI). According to the open field test, the impaired behavioral symptoms such as decreased traveled distance, low rearing activity and longer spatial probe time were nearly reversed to normal state by emodin treatment. Pathological examinations by light and electron microscopy showed that emodin reduced brain damage when compared to the control group after 24 h post-blast. On the other hand, emodin treatment after brain injury significantly decreased the release of NO and iNOS protein levels in cortical tissues in the control group. Therefore, this study suggested that emodin could ameliorate the bTBI-induced brain injury by preventing excessive NO production by inhibiting the activity and protein levels of iNOS [99].

Wang et al. [48] demonstrated the anti-inflammatory effects of emodin in a mouse model of asthma induced by ovalbumin (OVA) treatment. The intraperitoneal administration of 10 or 20 mg/kg emodin during the OVA challenge significantly mitigated the number of leukocytes present in the bronchoalveolar lavage fluid (BALF) and lung tissues as well as diminished IgE serum levels in comparison with OVA-challenged samples. Asthmatic induction triggered an excessive level of Th2 cytokines (IL-4, IL-5, IL-13) and eotaxin levels in BALF and, all of them were decreased by emodin treatment dose-dependently. In emodin-treated lung tissues lower mRNA levels of matrix metallopeptidase 9 (MMP-9), IL-4 and IL-5 and higher levels of the antioxidant HO-1 gene were observed when compared to the OVA group. Taken together, the inhibitory effect of emodin against allergen-induced inflammation seems a promising strategy for the treatment of asthma-related pathogenesis in the lung [48].

In the study by Cui et al. [98], the potential therapeutic effect of emodin on SAP-associated acute lung injury was investigated in Sprague-Dawley rats, using dexamethasone (DEX) as a positive control. Emodin significantly ameliorated the pancreatitis-induced increase in serum amylase activity and pre-B cell colony enhancing factor (PBEF) gene expression level which has been reported to exacerbate acute lung injury. On the other hand, while emodin protected the SAP-induced lung and pancreatic tissues from apoptosis, it also inhibited the excessive infiltration of polymorphonuclear neutrophils (PMNs) from peripheral blood into the lung by triggering the PMN apoptosis.

Yin et al. [100] evaluated the anti-inflammatory effects of emodin in LPS-treated RAW264.7 murine macrophages and the hepatoprotective effects against fulminant hepatic failure in an LPS/D-galactosamine (D-GalN)-sensitized mouse model. Oral administrations of 10, 30, 100 mg/kg emodin 24 h before LPS/GalN induction, decreased the mortality rate and repressed alanine aminotransferase (ALT) and aspartate aminotransferase [40] activities in a dose-dependent way when compared to the LPS /D-GalN induced mice. According to the results, several parameters such as extensive hemorrhage, necrosis, and neutrophil infiltration were also prevented by emodin treatment. This study showed that emodin restricted the phosphorylation of p-p38 and p-IκB proteins involved in MAPKs and NF-κB signaling pathways in LPS/D-GalN-sensitized mice. The LPS-induced mRNA and protein level of TNF-α was downregulated with the pre-treatment of emodin at a concentration of 100 mg/kg concerning D-GalN-sensitized mice and 3 and 10 μM in RAW264.7 cells. As consistent with the *in vivo* results, emodin showed a suppressive effect on NF-κB and activator protein 1 (AP-1) activation and downregulated the expression of toll-like receptor 4 (TLR4)/myeloid differentiation factor 2 (MD-2) complex reducing the production of pro-inflammatory mediators through MAPKs and NF-κB activation in LPS-stimulated macrophages [100].

In the study by Han et al. [101] the anti-inflammasome effect of emodin was investigated in an LPS-induced endotoxin shocked mouse model and bone marrow-derived macrophages (BMDMs). LPS-primed BMDMs were pre-treated with or without emodin (12.5, 25, 50 μM) for 1 h before NLRP3 inflammasome induction by nigericin and ATP stimulation for 1 h and silica crystals stimulation for 3 h. The study

demonstrated that emodin pre-treatment dose-dependently decreased the rate of cell death due to pyroptosis induced by LPS. Moreover, emodin treatment reduced the protein levels of cleaved IL-1β (p17) and caspase-1 (p10) found in the supernatants of BMDMs culture media. In addition, the intraperitoneal injection of 10 and 50 mg/kg of emodin increased the survival rate in the LPS-treated mice when compared to the control group. All of these results might be supportive of the protective potential of emodin against NLRP3 inflammasome-mediated-disease symptoms [101].

Pang et al. [102] displayed the anti-atherosclerotic and anti-inflammatory effects of emodin through the inhibition of homocysteine (Hcy)-induced C-reactive protein (CRP) expression in an atherosclerosis rat model and vascular smooth muscle cells (VSMCs). Hcy-treatment remarkably increased CRP expression in VSMCs which is a crucial inflammatory molecule for the progression of atherosclerosis. However, pre-treatment of VSMCs with emodin (0.1, 1, and 10 μM) significantly decreased the mRNA expression and protein levels of CRP in a dose-dependent manner. On the other hand, emodin did not induce any variation in the basal levels of CRP in non-treated cells. In addition, emodin treatment dose-dependently reduced the phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 when compared to the control group. Besides, Hcy–halted PPARγ expression was markedly upregulated by emodin. The daily oral administration of emodin at the dose of 40 or 80 mg/kg to Hyc-treated rats resulted in decreased serum CRP levels after four-week exposure. Moreover, emodin also downregulated CRP expression in the aortic walls of Hcy-treated rats [102].

The ameliorative effect of emodin on ulcerative colitis was studied in a dextran sodium sulfate (DSS)-mouse model and in a flagellin-stimulated HT-29 cell line [103]. In DSS-treated mice, the inflammatory cell infiltration, histological inflammation, and shortening of colon length together with the colon damage and loss of its integrity were relieved by emodin, administered at 5, 10 and 20 mg/kg of body weight daily for 2 weeks. Moreover, emodin treatment normalized the number of white blood cells at a 20 mg/kg dose when compared to the control group. In flagellin-induced HT-29 cells, while mesalazine -a prescribed ulcerative colitis drug- downregulated the NF-κB transcription without any significant change in TLR5 expression, emodin downregulated the TLR5/NF-κB signaling pathway by decreasing the flagellin induced protein and mRNA expression levels of TLR5, myeloid differentiation primary response 88 (MyD88), and NF-κB p65 proteins. Additionally, immunofluorescence staining evidenced that emodin at 20, 40 and 80 μM concentrations significantly reduced NF-κB p65 nuclear accumulation induced by flagellin in HT-29 and decreased the release of IL-8 [103].

The protective effect of emodin on myocardial ischemia/reperfusion (I/R) injury was also examined in rats and cardiomyocytes exposed to hypoxic conditions [104]. The percentage of the myocardial cells presenting distorted and blown morphology and the ratio of infarct size at the risk area was remarkably diminished by pre-treatment of emodin compared to the I/R group. Emodin also reduced the levels of gasdermin D-N domain (GSDMD-N), an executioner of pyroptotic cell death, by inhibiting the cleavage activity of caspase-1 in the left ventricle tissue subjected to I/R injury. As compatible with the results, primary cardiomyocytes treated with emodin at concentrations of 2.5, 5, and 10 μM 1 h before hypoxia exposure showed a significant reduction in the expression of GSDMD-N, p20 subunit of caspase-1 and secretion of IL-1β. The pyroptosis suppressive effect of emodin was found to be mediated by inhibiting the TLR4/MyD88/NF-κB/NLRP3 inflammasome pathway in hypoxia-exposed cardiomyocytes [104].

Recent research evaluated the anti-inflammatory properties of emodin in osteoarthritis using primary chondrocytes isolated from rats and a rat model in which the anterior cruciate ligament of the knee was cut [105]. Emodin treatment (0, 5, 10, 20, 45, 85 μM for 24 h) significantly reduced the elevated protein levels and gene expression of MMP-3, MMP-13 and ADAMTS-4 (ADAM metallopeptidase with thrombospondin type 1 motif 4) in chondrocytes induced by IL-1β. The treatment of animals with emodin (20,50,80 mg/kg from the fourth week after surgery until the end of the sixth week) resulted in a thicker and smoother surface layer of cartilage than that observed in the control group. The number of positive cells expressing MMP-3, MMP-13, ADAMTS-4 and iNOS was significantly reduced whereas collagen type II alpha 1 chain (COL2A1) was increased in cartilage after emodin treatment. In addition, the elevated serum levels of cyclooxygenase-2 (COX-2), and prostaglandin E2 (PGE2) induced by the osteoarthritis were reduced in the emodin-treated animals.

4.3. Neuroprotective activities

Neurodegenerative disorders are becoming an important threat to human health due to the complex mechanisms of diverse progressive CNS degeneration and neuronal loss [106–109]. Therefore, understanding their mechanisms completely and then, finding an effective therapeutic agent are seriously needed to cure affected patients [110–113].

4.3.1. In vitro studies

Park et al. [57] investigated *in vitro* the anti-neuroinflammatory effect of emodin (10, 20, 40, 80 μM during 24 h) and its regulatory role on the AMPK/Nrf2 signaling pathways in LPS-stimulated mouse primary microglia and BV-2 microglial cells. None of the tested concentrations showed any cytotoxicity in both primary microglia and BV-2 cells. Emodin treatment increased dose-dependently the phosphorylation of AMPK and the protein level of its upstream kinases, serine-threonine liver kinase B1 (LKB1) and Ca^{2+}/calmodulin-dependent protein kinase kinase-II (CaMKII). The Nrf-2 nuclear accumulation and the protein expression of HO-1 and NAD(P)H: quinone acceptor oxidoreductase 1 (NQO1) genes were also significantly upregulated after treatment with 40 μM of emodin in primary microglia by the activation of AMPK. Pre-treatment microglia cells with emodin for 1 h before LPS stimulation remarkably decreased the secretion of LPS-induced TNF-α, IL-6, NO, and prostaglandin E2 (PGE2) and has also inhibited the activation of iNOS and COX-2 enzymes in a process mediated by NF-κB inhibition. In addition, emodin also exerted neuroprotection by suppressing the LPS-stimulated activation of STATs, JNK, and p38 MAPK pathways [57].

The same group of researchers also investigated the emodin-induced neuroprotection against oxygen-glucose deprivation/reoxygenation (OGD/R) and the underlying mechanism *in vitro* in human neuronal SH-SY5Y cells [58]. Cells were pre-treated with 5, 10, 20, and 40 μM of emodin for 1 h and then, subjected to oxygen-glucose deprivation (8 h) before reoxygenation (24 h). Cytotoxic assays evidenced that emodin treatment was not toxic to SH-SY5Y cells up to 40 μM. Emodin pre-treatment significantly recovered the decreased viability and reduced the ratio of apoptosis induced by OGD/R in a dose-dependent manner. The molecular mechanism of the anti-apoptotic activity of emodin was associated with the decrease of the levels of the proapoptotic proteins cleaved PARP, cleaved caspase-3, and -9, p53, p21 and Bax. Furthermore, the expression of antioxidant genes (particularly, NQO1 and HO-1) were also increased by emodin dose-dependently in SH-SY5Y cells exposed to OGD/R concerning non-exposed cells *via* activation of the Nrf-2/ARE signaling pathway. Additionally, emodin treatment increased the phosphorylation of AMPK and GSK3β proteins. The presence of an AMPK inhibitor significantly decreased the emodin-induced protein expressions of p-GSK3b, HO-1, NQO1 as well as Nrf2 nuclear accumulation suggesting that these effects are mediated by the regulation of AMPK/GSK3β pathways [58].

Recently, the protective effects of emodin on zinc-induced neurotoxicity as a model of vascular dementia and Parkinson's disease was investigated by Liu et al. [59]. SH-SY5Y cells were pretreated with 0.5, 10, 20 and 50 μM of emodin for 24 h followed by 200 μM $ZnSO_4$ for 1 h. Emodin dose-dependently reversed the effects of $ZnSO_4$ by increasing cell viability and reducing the release of lactate dehydrogenase (LDH)

and apoptotic cell ratio. Moreover, emodin significantly decreased the expression of zinc transporter families (MT1, MT2, and ZnT-1) and inhibited the Zn^{2+} influx into SH-SY5Y cells despite the excess amount of Zn^{2+} in the medium. Pre-treatment with emodin significantly elevated the diminished intracellular levels of NAD^{+} and ATP induced with Zn^{2+} exposure. Furthermore, while Zn^{2+} exposure in these cells led to high-level ROS production and mRNA expression levels of ER-stress-related proteins including CHOP, GADD34, and ATF4, emodin markedly suppressed them. Additionally, pre-treatment of cells with emodin reduced the Zn^{2+}-induced phosphorylation of AMPK suggesting that the protective effects of the compound were mediated by the AMPK signaling pathway. A recent report investigated the protective effects of emodin (40 μM) against methylglyoxal-induced mitochondrial dysfunction in SH-SY5Y cells [60]. The pre-treatment with emodin for 4 h before the exposure to methylglyoxal for an additional 24 h recovered the reduced activity of the complexes I and V, the levels of adenosine triphosphate, and the loss of mitochondrial membrane potential. Mechanistic analysis indicated that the protective effects were mediated by the inhibition of the AMPK/Nrf2/HO-1 signaling pathway since the inhibition of this pathway suppressed the beneficial effects of emodin.

4.3.2. *In vivo studies*

In an *in vivo* study, the antidepressant-like activity of emodin against chronic unpredictable mild stress (CUMS)-induced behavioral deficiency was evaluated in mice for 6 weeks using fluoxetine as a positive control [61]. After the experimental procedure, the consumption of sucrose was remarkably reduced, the immobility duration was elongated and locomotor activities such as crossing, and rearing were weakened in the CUMS-exposed group concerning the control group. Emodin treatment (20, 40 and 80 mg/kg) for three weeks following CUMS exposure significantly reversed these symptoms and inhibited the progression of behavioral impairments in mice. Additionally, CUMS-induced plasma corticosterone levels were nearly returned to values similar to those of the control group after emodin treatment. Emodin significantly recovered the decreased mRNA expression of brain-derived neurotrophic factor (BDNF) and glucocorticoid receptor (GR) mRNA and protein expression levels in the hippocampus. A downregulation of hippocampal GR due to hypothalamic-pituitary-adrenal dysfunction and expression of brain-derived BDNF could be related to the mechanism of depression. Therefore, normalization of their expression levels could be a potential therapeutic target for the treatment of this disorder.

In an interesting study by Li et al. [62], it was evidenced that emodin can inhibit cerebral I/R-induced neurological deficit, increase the disrupted blood-brain barrier permeability and progression of cerebral infarction in I/R-induced SD rats. The protective effects of emodin were mediated by the downregulation of connexin 43 (Cx43) and aquaporin 4 (AQP4). Cx43 and AQP4 regulate the gap junction communication and cerebral water balance and consequently, the I/R-induced downregulation could participate in the progression of ischemic stroke leading to neuronal cell death and cerebral edema [114,115]. In the same study, the combined treatment of I/R- exposed rats with emodin and ginsenoside Rb1 showed synergistically higher neuroprotective effects in the same analyzes compared to the emodin-treated group [62].

Sun et al. [63] investigated the neuroprotective effect of emodin against Aβ-induced autophagy *in vitro* and *in vivo* studies (AβPP/PS1) double-transgenic mice were orally administered with 50 mg/kg per day of emodin for seven days after intracerebral ventricular injection of 10 nM of Aβ25–35. PC12 cells were treated with 10 μM of Aβ25–35 followed by 10 μM emodin and using 3-methyladenine (3-MA) as a positive control. The conversion of microtubule-associated protein light chain 3 (LC3)-I to LC3-II which triggers cell autophagy, was significantly increased in Aβ-induced AD mice. Emodin or 3-MA treatment significantly decreased the levels of Aβ25–35–induced lLC3-II, increased cell viability and reduced LDH leakage in both the animal and the cell model. Moreover, emodin treatment notably reversed the Aβ25–35-induced autophagy through increasing B-cell lymphoma 2 (Bcl-2) and ameliorating Beclin-1 expression, which was induced by amyloid-β 25–35 treatment. Using a PI3K inhibitor treatment, the results suggested that emodin could inhibit Aβ25–35 -induced neurotoxicity through the activation of the class III PI3K/Beclin-1/Bcl-2 pathway [63].

Ahn et al. [64] reported the neuroprotective effects of emodin against glutamate-induced oxidative toxicity in HT22 mouse hippocampal neuronal cells and a mouse model of photothrombotic ischemia. Non-cytotoxic concentrations of emodin (10, 20, and 40 mM) significantly increased the viability of HT22 cells and mitigated the release of LDH concerning glutamate-exposed cells. Pre-treatment with emodin, especially at 40 μM, prevented glutamate-induced apoptosis in HT22 cells *via* the PI3K signaling pathway and improved the neuronal cell viability *via* inhibiting the glutamate-induced DNA fragmentation in the sub-G1 phase. In the *in vivo* studies, intracortical injection of 3 nmol emodin in 1 μL for 15 min before ischemic insult operation markedly minimized cerebral infarct volume and escalated some motor skill scores when compared to the vehicle group. On the molecular basis, emodin significantly increased Bcl-2, pAKT, p-cyclic-AMP response element-binding protein (pCREB) and mature BDNF protein expression while inhibiting Bax and activating caspase-3 protein expressions [64]. The neuroprotective effects of emodin were also investigated by Leung et al. [65] in *in vivo* assays using PC12 cells exposed to oxygen-glucose deprivation and a rat model of I/R induced by right middle cerebral artery occlusion. Emodin treatment (1 and 10 μM for 24 h) increased cell viability and reduced ROS production and the release of glutamate which were altered by the ischemia/hypoxic conditions. Rats treated with emodin (15 mg/kg, i.p.) showed a reduced infarct size and a higher percentage of recovery in body asymmetry. At the molecular level, emodin increased the gene expression of Bcl-2 and glutamate transporter-1 (GLT-l) and reduced the levels of cleaved caspase-3 levels through modulation of the ERK1/2 signaling pathway. The results of research studies have shown that emodin can be considered a promising neuroprotective agent to prevent neurotoxicity/neuroinflammation, oxidative stress-induced neuronal loss, and progression of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) or cerebral ischemia.

4.4. *Anticancer*

Cancer comprises a group of diseases that cause abnormal cellular activities and growth in the body [8,116,117] The number of patients diagnosed with cancer grows day by day and negatively affects more than a third of the world population [118,119]. Researchers in the pharmaceutical field put their effort into the design and/or discovery of novel anticancer molecules [120] Although anticancer agents derived from synthetic molecules have shown promising therapeutic effects, drugs discovered inspired by natural products could have several advantages due to their minimized side effects and low toxicity [121,122]. Among these natural compounds, emodin has been investigated in a broad spectrum of preclinical test models to elucidate its anticancer activity.

4.4.1. *In vitro studies*

In the study by Lu et al. [123] the anticancer effects of emodin were investigated in A2780 and SK-OV-3 epithelial ovarian cancer cells. In this scope, two different cancer cell lines including A2780 and SK-OV-3 were treated with 0–80 μM of emodin followed by 24, 48, and 72 h incubation. The treatment with emodin (0–80 μM during 24, 48, and 72 h) evidenced dose and time-dependent antiproliferative effects. Moreover, the anti-invasive and anti-metastatic effects were evidenced with transwell migration and invasion assays. This study also illustrated the activity of the compound on the epithelial-mesenchymal transition. Protein analysis showed that the levels of epithelial markers such as E-cadherin and claudin were upregulated whereas the expression of mesenchymal markers including N-cadherin and vimentin were significantly downregulated in a dose-dependent manner. Additionally, it was

evidenced that the expression levels of Slug (a transcription factor) were also suppressed with emodin treatment. To clarify the mechanism of action, cells were transfected with siRNA to silence the key proteins for Slug and β1integrin-linked kinase (ILK). Interestingly, the results suggested that emodin suppressed the epithelial-mesenchymal transition of cancer cells through the ILK/GSK-3β/Slug signaling pathway [123].

Dong et al. [124] showed the anticancer effects of emodin (20, 40 and 80 μM of emodin for 24 and 48 h) in human hepatocellular carcinoma HepaRG cells. Emodin reduced the cell viability in a dose/time-dependent manner and promoted cell cycle arrest at S and G2/M phases when compared to non-treated cells. According to Annexin V/PI staining assay, emodin induced apoptosis in these cells and increased cell populations in the early and late stages of apoptosis. The mechanism of action was related to a decreased expression of the anti-apoptotic Bcl-2 and enhanced the expression of the pro-apoptotic Bax. Moreover, the expression levels of cleaved caspase-3, -9, and PARP were also significantly induced by emodin treatment when compared to the vehicle-treated group. All these findings suggested that emodin might induce apoptosis in hepatocellular carcinoma cells by the mitochondrial caspase-dependent pathway [124]. Ma et al. [125] investigated the effects of emodin (0–200 μM for 24 h) in the Caco-2 human colon carcinoma cells. Emodin decreased the cell viability in a dose-dependent manner suppressing the cell growth (IC_{50}) by 50 % at 30 μM. In addition, emodin treatment significantly elevated the percentage of cells in the early and late apoptotic stages and triggered cell cycle arrest at the G2/M phase. Besides, the effects of emodin on Bax/Bcl-2 protein expression levels and mitochondrial membrane potential revealed that emodin stimulated apoptosis *via* the mitochondrial pathway. Also, emodin downregulated the phosphorylated form of key proteins involved in the PI3/AKT signaling pathway, which is associated with tumorigenesis and cancer progression suggesting the potential use of emodin as an anticancer agent [125]. The anti-proliferative effects of emodin (1–100 μM for 48 h) were investigated in cervical cancer cells (HeLa) [126]. The inhibition of cell viability has been found in a dose-dependent way and the IC_{50} value was determined at 56.91 μM. Emodin treatment also increased the number of early and late apoptotic cells and significantly reduced the mitotic index. The cytometric analysis evidenced that emodin at 100 μM concentration disturbed the cell cycle by arresting the G2/M phase.

The anticancer effects of emodin (25, 50 and 10 μM for 24 h) were also investigated on MCF-7 human breast cancer cells [127]. The results evidenced that emodin at 50 and 10 μM was capable to inhibit cell proliferation and increase the apoptotic rate in a time and dose-dependent way. A molecular docking study reported that emodin could be fixed into the ATP binding pocket of the aryl hydrocarbon receptor (AhR) protein. Subsequent analysis showed that emodin can act as an AhR agonist, increasing its protein levels and those of its downstream target gene CYP1A1. These results were confirmed with an AhR inhibitor (CH223191) evidencing an increase in the survival rate of the cells. In the study carried out by Wang et al. [128], emodin (0–200 μM for 24 h) exerted anticancer effects in different kidney cancer cells -786-0, ACHN,Caki, and OS-RC-2 – but without toxic effects in n noncancerous cells HK-2. The mechanism of action was not related to the activation of apoptosis but was mediated by necroptosis. Thus, necroptosis-related proteins such as receptor-interacting protein kinase-1 (RIP1) and mixed lineage kinase domain-like pseudokinase (MLKL) were significantly elevated after emodin treatment in a mechanism related to increased ROS and the subsequent activation of the JNK pathway. In addition, emodin inhibited glycolysis *via* ROS-induced inactivation of the PI3K/AKT signaling pathway. An interesting line of research is the ability of emodin to act as a chemosensitizing agent. In this sense, it has been evidenced that emodin (110 μM for 48 h) sensitized breast cancer cells (MDA-MB-231 and MCF-7) to doxorubicin [129]. Emodin treatment enhanced the DNA-damaging properties of doxorubicin evidenced by increased levels of the marker of DNA damage γH2A. Moreover, emodin decreased the expression of AKT1, which has been reported to play a role in the resistance of breast cancer cells toward doxorubicin. The study by Peng et al. [130] also showed chemosensitizing effects of emodin to cisplatin in A549 (2–20 μM, for 48 h) and H460 (0.5–10 μM) non-small cell lung cancer cells. Co-exposure to emodin and cisplatin significantly inhibited cell proliferation and increased apoptosis more markedly than administered separately. The sensitization mechanism was mediated by the inhibition of P-glycoprotein (Pgp), a drug-resistant protein related to the efflux pump mechanism. Taken together, although additional studies are needed, suggests that emodin may be considered as an effective sensitizer of chemotherapeutic agents.

4.4.2. *In vivo studies*

The study by Manimaran and Manoharan [131] evaluated the chemopreventive effect of emodin (50 mg/kg three times a week for 14 weeks) against 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced buccal pouch carcinogenesis using a hamster model. At the end of the administration period, animals were sacrificed and the tissues were saved for biological assays. The protein levels of apoptotic markers were determined by Western blot analysis. At the end of the experimental procedure, the expression level of Bcl-xL was significantly increased in the buccal mucosa of hamsters treated with DMBA, whereas emodin treatment led to a significant reduction in the expression level. Moreover, emodin upregulated the expression levels of p53, Bax, Bcl-2–associated death protein (BAD), caspase-3 and -9 which were downregulated by DMBA. Histopathological tests confirmed that emodin-treated groups did not display any cancer formation on their buccal pouches while the animals treated with only DMBA exhibited significant tumorigenesis [131].

In a study carried out by Lin et al. [132] the anticancer activity of emodin was investigated in both SMMC-7721 hepatocellular carcinoma cell line and animal models. Emodin treatment (0, 10, 25, 50, 100 and 200 μM for 12, 24 and 48 h) inhibited in a dose and time-dependent manner SMMC-7721 cell proliferation in the control group. In the same cell line, flow cytometric analysis indicated that the increase in emodin concentration was highly correlated with the increased percentage of apoptotic cells. Immunoblot analysis showed that emodin did not alter the total expression of ERK, p38, and JNK, however, the phosphorylated form of ERK and p38 were significantly upregulated in a time-dependent manner while the level of p-JNK was suppressed. Additionally, the levels of p-Akt were decreased but without any change in total Akt expression. Furthermore, the expression levels of cleaved caspase-3 and -9 were significantly upregulated in the emodin-treated SMMC-7721 cells. The analysis of the ameliorative effect of emodin was investigated in BALB/c-nu nude mice subcutaneously inoculated with 5×10^6 SMMC-7721 cells. When the size of the tumor reached 75–100 mm^3, mice were divided into three different groups and exposed to vehicle, 25 or 50 mg/kg of emodin daily for two weeks. Emodin treatment inhibited tumor growth in mice in a dose-dependent manner, without significant effects on the total body weight of the mice. The significant reduction of Ki-67 and proliferating cell nuclear antigen (PCNA) protein levels supported the antiproliferative effect of emodin in animal models. To sum up, this study demonstrated the potential ameliorative effects of emodin in the treatment of hepatocellular carcinoma [132]. Su et al. [133] reported the effectiveness of emodin in the A549 and H1299 human non-small lung cancer cell lines. Similar to the results mentioned above, the 0–80 μM of emodin treatment resulted in a decreased proliferation rate in a dose-dependent manner as well as an increased apoptotic rate. Moreover, small interfering RNA (siRNA) knockdown was utilized to determine the role of tribbles homolog 3 (TRIB3). The obtained results indicated that the silencing of this gene led to a reduction in the apoptotic cell rate. Both TRIB3 gene silencing and inhibition of ER stress by 4-phenylbutyrate (4-PBA) led to the inhibition of NF-κB signaling and, significantly decreased the apoptotic effect of emodin. Therefore, all these findings suggested that emodin exerts its apoptotic effects in a process mediated by ER stress and the activation of

the TRIB3/NF-κB pathway in lung cancer cells. In the same study, BALB/c nu/nu nude mice were subcutaneously injected with a mixture containing A549 cancer cells (2.5×10^6) and growth factor-reduced matrigel. After 24 h, animals were divided into 4 groups, and each group was subjected to vehicle alone (20 mL/kg), 4-PBA alone (20 mg/kg/day); emodin (50 mg/kg/day) and emodin with 4-PBA, once per day. Emodin treatment significantly reduced the tumor growth when compared to the control groups whereas the effect was inhibited by co-exposure to 4-PBA [133]. The anticancer effects of emodin (2.5 and 5 mg/kg for 7 days) were investigated in a rat model implanted with CC-531 colon cancer cells [134]. 21 days after the final dose of emodin, a significant decrease in intraperitoneally-induced tumor weight was observed concerning the control group. Similar protective effects were also observed after the treatment with BTB 14431an in-silico homolog to emodin but at a lower dose of 0.3 mg/kg. A recent study investigated in a mouse model the preventive effects of emodin against post-surgery metastatic recurrence of breast tumors in the lungs [135]. Emodin (40 mg/kg for 7 days) significantly decreased the metastatic recurrence of breast cancer after surgery in the lungs by reducing the formation of epithelial-mesenchymal transition (EMT) and cancer stem cell (CSC). The mechanism of action was associated with the inhibition of both canonical and noncanonical pathways of transforming growth factor (TGF)-β1 signaling.

4.5. Anti-diabetic

4.5.1. In vitro studies

Jung et al. [136] conducted an *in vitro* study to investigate the anti-diabetic potential of various constituents of *S. obtusifolia* including emodin. The inhibitory effects of the different compounds were investigated against protein tyrosine phosphatases 1B (PTP1B) which decreases insulin sensitivity and α-glucosidase which plays a critical role in postprandial hyperglycemia *via* increasing glucose absorption. The highest PTP1B and α-glucosidase inhibitory activities were detected for emodin and alaternin. The effect of emodin on glucose uptake was investigated in insulin-resistant HepG2 cells. Cells treated with emodin at non-cytotoxic concentrations (3.125, 6.25, and 12.5 μM) for 24 h followed by insulin 100 μM for 30 min had an improved insulin-induced uptake of glucose in the control group [136].

4.5.2. In vivo studies

In the study by Arvindekar et al. [137] the anti-diabetic potentials of five 1,8-dihydroxyanthraquinones (DHAQs) - aloe-emodin, chrysophanol, emodin, physcion and rhein - isolated from *Rheum emodi* wall. ex Meissn were investigated in a diabetic rat model induced by streptozotocin administration. Each DHAQs was administered to diabetic rats at a dose of 2 mg/kg bw by the intragastric way. The oral glucose tolerance test evidenced that all of the compounds significantly reduced hyperglycemia in the order of emodin, rhein, emodin, physcion, and chrysophanol in the control group. Nevertheless, only emodin showed effective α-glucosidase inhibitory effects at lower concentrations similar to acarbose, the positive control [55]. In addition, diabetic rats were also subjected to 3 mg/g bw of maltose load reporting that emodin treatment decreased the blood glucose level and inhibited maltase activity more effectively than the positive control. Since starch is an essential component of the human diet, the inhibition of maltase and alpha-glucosidase enzymes by emodin is an interesting point to explore the anti-diabetic effect of this compound [137]. In a study conducted by Li et al. [67] the beneficial effects of emodin on glucose metabolism were investigated in high fat-diet (HFD)-fed mice. Emodin administration (40 and 80 mg/kg) to obese mice daily for six weeks significantly decreased the fasting blood glucose level and enhanced insulin sensitivity in obesity-induced mice as compared to the control group [67]. Yu et al. [138] investigated the protective effects of emodin against non-alcoholic fatty liver disease in zebrafish models induced by egg yolk powder administration. Emodin treatment significantly reduced fish body weight, body mass index, histological alterations and lipid accumulation in the liver. Emodin also reduced hepatic lipogenesis in a mechanism of action mediated by the induction of the AMPK signaling pathway evidenced by a notable increase in p-AMPK and the gene expression of downstream target genes including PPARα, acyl-coenzyme A oxidase 1 (ACOX1) and carnitine palmitoyltransferase 1a (CPT-1a).

4.6. Immunosuppressive

Abnormally activated immune cells can cause many different types of autoimmune disorders such as rheumatoid arthritis or multiple sclerosis, therefore, the regulation of apoptosis in these cells is a crucial point to treat or prevent the severe pathologic symptoms of these diseases [139–142].

4.6.1. In vivo studies

According to the study by An et al. [143], splenocytes isolated from C57BL/6 mice were co-treated with emodin at non-cytotoxic concentrations of 2.5, 5, 10, and 20 μM in the presence or absence of 5 μg/mL of ConA, a T cell activator, for 72 h. Emodin treatment significantly decreased cell viability and proliferation in splenocytes stimulated with Con A. Surface-flow cytometry assay proved that emodin inhibited the proliferation and differentiation of specific B cells and the conventional dendritic cells (cDC). Accordingly, 20 μM emodin treatment significantly reduced the population of CD45R/B220$^+$ and CD19$^+$ CD69$^+$ cells as compared to Con A-treated controls. The population of IL-17 and IL-10 cytokine-producing T cells were also decreased by emodin treatment. Therefore, emodin may be developed as an immunosuppressive agent in case of immune activation, autoimmune disorders even in organ transplantation [143].

Despite the availability of various immunosuppressant drugs such as cyclosporine A (CsA), allograft rejection is still one of the huge problems in organ transplantation due to immune responses. In the study by Qui et al. [144], the immunosuppressive capacity of emodin was investigated in skin transplantation in a C57BL/6 mouse receiving a skin graft from a donor Balb/C mouse. Emodin (10 mg/kg bw), CsA (20 mg/kg bw) or a combination of both agents were administered to the animals for 4 weeks or until graft rejection. Emodin treatment significantly prolonged the skin allograft survival time concerning the non-treated control and also inhibited CD3$^+$ T cell infiltration while promoting CD4$^+$FoxP3$^+$ and CD8$^+$CD122$^+$ regulatory T cells generation. Interestingly, the reducing effect of CsA on CD4$^+$Foxp3$^+$ cells in draining lymph nodes was remarkably reversed in mice that received both emodin and CsA. Moreover, emodin treatment suppressed the alloantibody production and prevented the posttransplantation dendritic cell maturation. Finally, emodin was capable to repress T cell proliferation and mTOR signaling in an *in vitro* assay [144]. Consequently, based on these results, emodin may be considered a promising immunosuppressive therapeutic agent.

5. Emodin in clinical trials

One of the main problems of plant-derived bioactive compounds is that although there is much *in vitro* or preclinical evidence of their therapeutic potential, clinical trials are scarce or give inconclusive results. The case of emodin is not an exception, and to date, there are practically no studies in humans that validate the results observed in other types of studies.

A clinical trial (NCT00801268) was designed to investigate the efficacy of *Tripterygium wilfordii* Hook. f. extract (60 mg/d) using emodin (100 mg/d) as an active comparator for autosomal dominant polycystic kidney disease (ADPKD). However, although the study began in 2008, to date there are no data available on the results obtained. The only preliminary data are those of an uncontrolled trial, where 9 proteinuric ADPKD patients were treated with T. *wilfordii* for 6 months [145]. The results evidenced that the treatment stabilized kidney function and

reduced proteinuria. In a second clinical trial (ChiCTR-TRC-14004653) the effects of emodin were investigated against peripheral blood neutrophil apoptosis in patients with acute pancreatitis. As in the previous study, there are no published data on the results obtained.

On the other hand, several studies have analyzed the possible effects of various plant extracts rich in emodin, but as they have a complex composition, they do not allow us to determine whether the results obtained are derived from emodin or surely, from the combination of the different bioactive compounds [146,147].

6. Therapeutic limitations and clinical gaps of emodin

In the last decade, an emerging trend in therapeutically active small molecules derived from natural sources has accelerated the efforts of researchers to elucidate the beneficial effects and mechanisms of action [148,149]. The plethora of pharmacological activities exhibited by emodin suggests that it could be an effective therapeutic option for the long-term prophylaxis and treatment of several disorders [150]. However, studies are lacking in the context of emodin contraindications which may be a limitation for its therapeutic use. In this sense, it was reported that emodin can impair embryonic development in mice and exert inhibitory effects on sperm motility dose-dependently but not on its viability *in vitro* assays [151,152]. Dose- and time-dependent toxicity of emodin has also been detected in kidney and liver cell lines [153].

The clinical application of emodin is also limited due to its very reduced bioavailability (less than 3 %) [20]. Once ingested, emodin rapidly undergoes a phase II metabolism in intestinal epithelial cells to form its glucuronide form by the action of UDP-glucuronosyltransferases, while the original form of emodin was almost undetectable *in vivo*. This glucuronidation is directly related to the low bioavailability of the compòund due to the action of the multidrug resistance-associated protein that is responsible for the efflux transport and, consequently, for the excretion of emodin glucuronide in the intestine [35].

Interestingly, combined administration of emodin and piperine has been observed to clinically improve emodin pharmacokinetics, increasing 221 % of the area under the curve (AUC), 258 % the maximum concentration (C_{max}), and decreasing 230 % the clearance related to inhibition of glucuronidation [28]. The mechanism by which the bioavailability of emodin is increased has not been elucidated. Also, it has been evidenced that emodin orally administered similarly to other constituents of processed *R. multiflora* products presented lower bioavailability compared to the raw product in a rat model [154].

Paradoxically, emodin exerts an antioxidant and pro-oxidant action and has also been reported to exhibit protective and toxic effects on rat glioma cells. Although in an *in vitro* assay, the mutagenic activity of emodin has been established at concentrations of 80 and 120 μg/mL through thymidine kinase gene mutation analysis, no consensus on its mutagenic activity has been attained [155]. A two years study carried out by the National Cancer Institute [156] did not show clear evidence of carcinogenicity in rats.

An excess of emodin due to its laxative effects causes intestinal pain and severe diarrhea with subsequent electrolyte imbalance and dehydration [157]. Therefore, treatment should begin when symptoms appear, with special attention to electrolyte levels, mainly potassium, especially in aged patients and children.

7. Summary and outlooks

Emodin, as part of various formulations of plant extracts, has been used since ancient times in traditional Chinese medicine. Recent studies have investigated its potential therapeutic effects against chronic diseases such as cancer, diabetes, neurodegenerative diseases or chronic inflammatory diseases. Previous *in vitro* and *in vivo* pharmacological studies have highlighted the therapeutic potential of emodin. In this current review, data on new molecular mechanisms of action and signaling pathways of emodin have been updated, mainly regarding its antioxidant, anti-inflammatory, anti-cancer and neuroprotective capacity. These updated studies are important for highlighting the current evidence-based therapeutic efficacy of emodin. The main limitations we find in terms of emodin derive from its low bioavailability derived due to its extensive metabolism and the absence of clinical studies to allow its application in clinical practice. Although preclinical pharmacological studies have highlighted the molecular mechanisms and defects of emodin signaling pathways, there is still no clear evidence of mechanisms of action. Chronic use of emodin could lead to potential toxicity, especially when used in high doses.

In conclusion, although emodin can be considered a potentially promising therapeutic agent against a large number of diseases, it is necessary to conduct additional human clinical trials to confirm its efficacy and safety. Also, new pharmaceutical nanoformulations to increase bioavailability and reduce toxicity are needed. In light of these data, emodin is a biomolecule with a therapeutic future.

CRediT authorship contribution statement

J.S.-R. contributed to designing the review. J.H.-B., S.K., K.P., A.P. M., M.M.-M., D.S.A., Z.Y., A.Y., contributed to collecting the paper from online databases. J.S.-R., A.S., M.M., C.H., D.C., W.C.C. contributed to writing, editing, and revising the manuscript. J.S.-R., W.C.C, C.H., A.S., and D.C. made the revision and final edits on the manuscript. J.S-R, C.H., D.C. supervision. All the authors read and approved the final version of the manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Data Availability

The authors are unable or have chosen not to specify which data has been used.

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