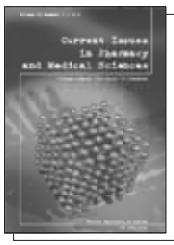

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Imperatorin – pharmacological effects and possible implication in pharmacotherapy

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ABSTRACT

In the presented work, the biological activity of coumarins, especially that of imperatorin, is described. The natural sources of coumarins and possible implication in pharmacotherapy is also shown.

Keywords: imperatorin, photosensibilizing activity, neuractivity, myocardial activity, anticancer activity, antioxidant activity, anti-inflammatory activity, antibacterial activity, anti-hiv-1 activity

INTRODUCTION

The prevalence of civilized diseases such as hypertension or other neurodegenerative disorders is increasing [10, 35, 48], and the more popular medicines used in the above-mentioned epidemiological problems are based on synthetic chemical structures produced in many laboratories all over the world. Unfortunately, pharmacological resistance and many adverse results (such as hepatotoxicity) are now widely present in the treatment of many common diseases. Because of the obligatory recording of drug side effects in the health systems of most developed countries, the very evident recognition of side effects boosts the necessity for a search for alternative solutions for present treatments. The drugs of choice will be those which not only reduce the symptoms of the disease, but which also will avoid the risk factors of the consequential ongoing changes in the human body. What is more, an additional target of scientific research will be in finding ways of mitigating the side effects of synthetic drugs. These aims have lead current researchers towards determining new solutions for several common treatments, in the hope that these will avoid the current adverse results, as well as the build-up of tolerance, and provide a wide spectrum of efficacy. Plant species, medicinal herbs and natural compounds play a crucial role in the placing of such new medicines upon the market. The aim of this work is therefore, to present information in regard to the natural sources of coumarins (especially furanocoumarins), and to show the biological activity of these compounds, laying emphasis on imperatorin.

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THE SOURCES OF COUMARINS

Natural sources provide numerous bio-active compounds that strengthen the potential of traditional medicine in treating many diseases and disorders. Representatives of genus *Angelica* as *Angelica dahurica*, *Angelica archangelica*, *Angelica amurensis*, *Angelica anomala*, *Angelica gigas* are widely used in ethnomedicine in China, Korea, Thailand and Russia to treat abdominal pain, hysteria, bleeding and menstrual disorders. In these countries, the biological activity of these plants as relaxants and sedatives have been known for many centuries. The strategy for current research and development is thus, to discover the natural compounds contained within these plants and identify their pharmacological activity. From previous research, it is known that plants of the *Angelica* species, especially their roots and fruits contains a wide range of furanocoumaris: e.g. isoimperatorin, imperatorin, phellopterin, oxypeucedanin hydrate, nodakenin, 3'-hydroksymarmesinin, also polyacetylenes, falcarindiol and octadeca-1,9-dien4,6-diyn-3,8,18-triol. Moreover, the roots of *Angelica sp*. are prescribed in China medicine as analgesics and sedatives. In our research, the coumarins from *Angelica dahurica* were studied for their chemical structures. This large group belongs to the family of phenolic compounds with diverse substituents, and which offer various pharmacological activities [9].

IMPERATORIN

One of the more widely researched natural compounds is imperatorin. This comes with a very wide spectrum of biological activity on the body system. Imperatorin is isolated from many plants e.g. *Radix Glehniae, Radix Angelicae dahuricae, Rhizome Seu, Radix Notoptygerii,* and is also present in different herbs commonly used in cuisine (parsley and fennel). In addition, imperatorin can be isolated from the

fruits and roots of several *Angelica* species, *Aegle marmelos* [50], *Heracleum rapula* [23], *Radix Glehniae* [22] and also from *Cinidum monnieri* [23, 30].

The biological activity of imperatorin was discovered as being a pharmacological effect upon the central nervous system. Moreover, it has some activity in the insulin fat cells [19, 20]. Imperatorin can also inhibit -aminobutyric acid degradative enzymes and elevate the neurotransmitter -aminobutyric acid levels in the central nervous system [9]. Plus, it inhibits HIV-1 infection [41] and induces oxidized-low density, lipoprotein-induced, enhanced intercellular adhesionmolecule-1 expression in U937 foamcells [49]. Furthermore, imperatorin antagonizes the proliferation of bovine cerebral microvascular smooth muscle cells induced by the tumor necrosis factor [50]. In lipopolysaccharide-activated mouse macrophages models, imperatorin inhibits nitric oxide and prostaglandin E2 production. What is more, it inhibits inducible nitric oxide synthase protein expression, cyclooxygenase-2 and microsomal prostaglandin E synthase expression [3; 29. 54]. Imperatorin can also inhibit bacterial growth and it exhibits a significant antitumoral property and an anticoagulant activity [18]. Finally, Imperatorin has also been studied for its anti-inflammatory and antitumoral potential [3. 13, 16].

PHOTOSENSIBILIZING ACTIVITY

Psoralens (e.g impeatorin), in combination with UVA radiation (320-400 nm), are used for the treatment of skin and autoimmune diseases, and the photochemical reactions of psoralens with DNA are well characterized, having been studied for many years. From *in vitro* DNA-photochemistry, it has been shown that the more frequently occurring adducts are those with thymine, followed by cytosine–psoralen adducts. A psoralen–adenine photo-adduct has also been characterized after *in vitro* photo-reactions with adenine, but it has not yet been shown to occur in DNA isolated from cells irradiated in the presence of psoralens [51]. In guinea pig skin treated topically with PUVA, psoralen binds preferentially to the keratine layer, to the cell membranes and to the intercellular matrix [34]. After UVA irradiation, the photo-adducts are localized in the same sites, whereas the photo-adducts in nuclear DNA are revealed only after special pretreatments with ethanol, alkali or proteolytic enzymes. This suggests that psoralen photoproduct formation in the membrane imparts significant damage to the cell that is different from that induced in the cell nuclei [52]. Additionally, in studies of the subcellular fractions of rat epidermis after treatment with psoralens and UVA, it was found that 17% of the psoralen was bound to DNA, while a substantial amount was also bound to proteins (57%) and lipids (26%) [4].

The photo-oxygenation of the imperatorin shows that the imperatorin hydroperoxides I and II cause oxidative DNA damage in cells. Moreover, imperatorin induces photosensitized damage of human erythrocytes. The mechanism of this process was studied with emphasis on the role of type IV reactions that involve photo-oxygenation. Data shows that the action of these photoproducts on erythrocytes brings about several types of cell damage, *i.e.* an increase in membrane permeability, as well as Hb damage. In addition, recently it was found that imperatorin hydroperoxides are very efficient suppressors of delayed-type hypersensitivity reactions in mice. These results imply that hydroperoxides I and II are potential chemotherapeutic agents for hyperproliferative T cell-mediated diseases. These peroxides are produced under UVA irradiation of cells, in the presence of imperatorin. Thus, imperatorin is a potential photochemotherapeutic agent for psoralen plus UVA (PUVA) therapy, operating by a type IV mechanism [38].

It is now well established that oral administration of furocoumarins is associated with gastrointestinal side-effects, and increases the risk of serious complications such as carcinogenesis or glaucoma. To reduce the side-effects of systemic PUVA, topical PUVA therapy has been developed. Currently available formulations for topical use are solutions, emulsions and creams, however, they do not provide good transdermal permeability of furocoumarins and do not allow its penetration to deeper layers of the skin. Higher concentration of furocoumarins delivered to the skin allows a reduction in UVA radiation dosage and side effects. Therefore dendrimers with furocoumarins can be used to improve solubility and bioavailability, and are able to release this drug in a controlled manner [5].

NEURACTIVITY

Imperatorin, together with another coumarin – falcarindiol, were prescribed so as to inactivate gamma-aminobutyric acid transaminase (GABA – T) in dose- and time-depended manners. Mammalian tissues of the central nervous system contain gamma-aminobutyric acid (GABA), and Glutamate decarboxylase (GAD) and GABA tramsaminaze (GABA-T) control the GABA concentration in the brain. GABA – T also catabolizes gamma-aminobutyric acid to succinic semialdehyde in transamination reactions. Succinic semialdehyde dehydrogenase (SSADH) is responsible for oxydation of succinic semialdehyde to succinate:

$$\text{GABA + alfa- ketoglutarate} \xrightarrow{\text{GABA - T}} \text{succinic semialdehyde (SSA) + glutamic acid qSSA + } NAD^+ + H_2O \xrightarrow{\text{SSADH}} \text{succinate + NADH}$$

According to Perry et al., a low level of GABA in the brain is the cause of diverse neurological effects. Among these are Parkinsonism, epilepsy, convulsions, seizures and Huntington's disease. In convulsive disorders, the chemical analog of GABA is used in the mechanism of irreversible inhibition of GABA –T, which is transformed to an active metabolite to bind the active site of the enzyme [36]. The vitro study of Choi et al. showed that imperatorin is an irreversible inhibitor of GABA –T. In the cited study, the isolation of coumarins and polyacetylenes was brought about by an 80% aqueous methanol extraction of material at room temperature overnight. For their work, Choi et al. used

the powdered root of *Angelica dahurica* from Korea. Their isolation afforded isoimperatorin, imperatorin, falcarindiol and phellopterin [9].

Luszczki et al discovered that imperatorin, in a dose-dependent manner, increases the threshold for electroconvulsion in mice. The greatest anticonvulsant effect produced by imperatorin in the maximal electroshock seizure threshold test was observed at 30 min after its systemic (i.p.) administration. The anti-electroshock action of imperatorin at 15, 60 and 120 min after its administration was less expressed than that of imperatorin injected 30 min before the maximal electroshock seizure threshold test [25]. It is important to note that toxicological in vivo studies have revealed that imperatorin administered chronically up to 70 mg/kg/day and singly up to 200 mg/kg produced no toxicity in rodents [21]. Imperatorin inhibits in both time- and concentration-dependent manners, GABA-transaminase activity. In addition, it displays affinity to the GABAA-benzodiazepine receptor complex as a partial agonist [12]. It is widely accepted that drugs inactivating GABA-transaminase contribute to the increase in GABA content into the synaptic clefts and thus, the drugs exert the anticonvulsant activity in both experimental and clinical studies [11].

Another study led by Luszczki and co-workers in 2007, demonstrated that the GABA activity of imperatorin is explained by the mechanism of imperatorin acting in specific modification of functional groups in the active site of GABA-T. The other specific results of the study showed that imperatorin has selective potentialisation effect on such anti-epileptic drugs as carbamazepine, phenobarbital, phenytoin and valproate. The methodology was based on the treatment of the above-mentioned conventional anti-epileptic drugs by imperatorin in the mouse maximal electroshock seizure model (MES) in dose and time depended manners. The ED_{50} – median effective doses required to protect 50% of animals tested against electroshock-induced seizures of carbamazepine, phenobarbital and phenytoin were explicitly decreased in the study by imperatorin addition. Interestingly, the anti-epileptic effect of valpronate was not improved by imperatorin at different, enhanced doses.

The authors also measured the influence of imperatorin on the brain concentration of phenytoin and phenobarbital, and this showed no significant changes in their concentration after imperatorin administration. This is in comparison to carbamazepine administration, in which, phenytoin and phenobarbital concentration was increased by 85%, from 1,260 to 2,328 μg/ml. The hypothesis to explain this is that imperatorin addition influences changes in the permeability of the blood/brain barrier. As to the specific valproate results, these were explained as engendering many, varied mechanisms of action on the central nervous system. [26]. In a subsequent investigation, Luszczki et al. demonstrated imperatorin potentialisation on lamotrigine. After 30 min of the 50mg/kg imperatorin administration, the potential of lamotrigine was enhanced by reducing ED_{50} by 60%, from 6.11 to 2.47 mg/kg. However, this dose of imperatorin did not influenced the brain concentration of lamotrigine, probably by way of the same explanation as phenytoine and phenobarbital – through various pharmacodynamic processes [27].

Cholinesterase inhibitors have become one of the more commonly prescribed drug classes for the treatment of Alzheimer's disease (AD) – a disease which is characterized by an abnormal shortage of the neurotransmitters acetylcholine (ACh) and butyrylcholine (Bch), in the brain [32]. Since ACh and BCh are hydrolyzed by acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), inhibition of the cholinesterases is important for increasing ACh and BCh levels. On the other hand, oxidative injury caused by free radical formation and iron accumulation has been revealed to be additional factors in AD pathogenesis [2]. Imperatorin, xanthotoxin, and bergapten were tested for their anticholinesterase and antioxidant activities in the same manner as was methanolic fruit extract (*Angelica officinalis* L. grown in Poland) and furanocoumarin fractions. Our data showed that imperatorin, xanthotoxin, and bergapten display a high inhibition (over 80%) against BChE at the tested concentrations. However, the furanocoumarin fraction and major three coumarins did not possess anti-radical activity against both 2,2-diphenyl-1-picrylhydrazyl (DPPH), N,N-dimethyl-p-phenylendiamine (DMPD), yet, they exhibited a moderate level of ferrous ion-chelation capacity, ferric-(FRAP) and phosphomolibdenum-reducing antioxidant power (PRAP). In a ferrous ion-chelation test, imperatorin also had better activity than did the other two coumarins tested [43].

In another study, the methanol extract of the roots of *Angelica dahurica* showed a high AChE inhibition, which led to isolation of isoimperatorin, imperatorin, and oxypeucedanin as the active compounds [17; 47]. These three coumarin derivatives inhibited AChE in a dose-dependent manner. The most effective of these was found to be imperatorin, as this caused a reversible inhibitory action against AChE. However, Sigurdsson and Gudbjarnason (2007) report that the AChE inhibitory activity of both an ethanol extract prepared from the seeds of *A. archangelica,* as well as xanthotoxin, had more potent AChE inhibition than that of imperatorin. Therefore, the cholinesterase inhibitory effect of the furanocoumarin fraction of *A. officinalis* can be attributed to coumarins. It is thus felt that further research on Coumarins might be suggested to determine their possible beneficial effects for the treatment of AD [44].

MYOCARDIAL ACTIVITY

Zhang et. al, (2010) revealed that, *in vivo* and *in vitro,* furanocumarins inhibited myocardial hypertrophy. In this study, cardiac myocytes were exposed to angiotensin II in the presence of imperatorin (3 μM, 30 μM, 10 μM) for 48h. The rats were 14-15 week old and they were placed into three groups: A, B, C treated with imperatorin. The two first groups were given a placebo – 0.5% sodium carboxymethyl cellulose. The Tail-cuff method was used to measure heart rate and blood pressure. Five myocardial slices chosen randomly from each group were used to measure myocardial

and perivascular fibrosis and cell surface area. The results of the study showed that imperatorin had a positive influence on blood pressure. On comparing the pharmacokinetic of verapamil to imperatorin, these investigators demonstrated a similar shape of chromatographic retention peak on the elution curves in cardiac muscle/CMC model.

The cell culture model used in the study allowed the investigators to control the size of myocardial cells. In their work, four comparative groups were established: control, angiotensin II, angiotensin + low-dose of imperatorin and angiotensin II + high dose of imperatorin. The results of their work showed that Imperatorin suppressed the increase of cell growth induced by angiotensin II in a significant way. The extent of myocyte hypertrophy was also reduced in this study. This was made evident in the histological test. Using the C group, the study revealed that imperatorin decreases pathological cardiac fibrosis and prevents its consequences (heart failure). This effect was achieved with the higher dose of imperatorin. The above-mentioned *in vitro* and *in vivo* experiments show that imperatorin plays an important role in myocardial hypertrophy by way of its pharmacological activity on cardiac muscle/CMC. Zhang et al. proposed a hypothesis that imperatorin, in comparison with verapamil, can act on the calcium channel by binding with the cardiac muscle. This idea is interesting and is worth further investigation [53].

In the study done by He et al. [15], imperatorin at a more than a 10 ěM concentration-dependent dose, relaxed rat mesenteric arteries that were pre-contracted by potassium chloride (KCl) and endothelin-1, as well as human omental arteries pre-contracted by noradrenaline. Removal of the endothelium did not affect the imperatorin-induced relaxant responses, suggesting that the vasodilatation effect is independent of the endothelium. The inhibitory effect of imperatorin is thought to occur mainly via the voltage dependent calcium channel and possibly the receptor operated calcium channel. However, the â-adrenoceptor, ATP-sensitive potassium channel and inwardly rectifying potassium channel were not involved in the vasodilatation, whereas the blockage of the calcium-activated potassium channel with tetraethylammonium had an effect. In a Ca2+-free medium, imperatorin concentration-dependently depressed the vasoconstrictions derived from noradrenaline and CaCl2, and brought about a decreased contractile response induced by caffeine. This indicates that it has a role in inhibiting extracellular Ca2+ influx and intracellular Ca2+ release from the Ca2+ store. These results suggest that imperatorin induces vasodilatation by possible mechanisms that inhibit the voltage dependent calcium channel and the receptor-mediated Ca2+influx and Ca2+release. The opening of the calcium-activated potassium channel and a competitive antagonism of 5-HT receptors may also contribute to this vasodilatation effect [15].

ANTICANCER ACTIVITY

Apoptosis, or programmed cell death, is considered a vital component of various processes, including normal cell turnover, proper development of the immune system, and chemical-induced cell death [42]. Inappropriate apoptosis is a factor in many human conditions including neurodegenerative diseases, ischemic damage, auto-immune disorders, and many types of cancer. Apoptosis is therefore, currently considered an efficient strategy for cancer therapy. [6]

There are conflicting reports of the effects of imperatorin on carcinogenesis. Imperatorin was mutagenic and induced transformation of mouse fibroblast cell lines, whereas it provided inhibiting effects on mutagenesis and carcinogenesis induced by various carcinogens. As possible mechanisms for anticarcinogenic agents, suppression of cell proliferation and induction of apoptosis are regarded as important factors, together with the decrease of metabolic activation, increase of detoxification, antioxidative properties, inhibition of DNA adduct formation and oncogene activation [8; 21]. According to Pae et al., imperatorin at cytotoxic micromolar concentrations, can trigger apoptosis of HL-60 cells. The HL-60 cells treated with imperatorin underwent internucleosomal DNA fragmentation and nucleus morphological changes characteristic of apoptosis. What is more, flow cytometric analysis showed that hypodiploid nuclei of HL-60 cells were increased after treatment with imperatorin. In addition, imperatorin caused a time-dependent increase in caspase-3 activation, an important key executioner of apoptosis. It was also demonstrated that imperatorin induces an alteration in the mitochondrial membrane potential and the release of cytochrome c from mitochondria into cytosol, and thus, the activation of caspase- 9 [33].

Cai et al. demonstrated that several naturally occurring coumarins, to which humans are routinely exposed, are potent inhibitors of the metabolism and metabolic activation of benzo[*a*]pyrene B[*a*]P and 7,12-dimethylbenz[*a*]anthracene DMBA in cultured mouse keratinocytes. Several of these compounds, upon further examination, also demonstrated some selectivity toward the formation of specific metabolites of both hydrocarbons. These results support the involvement of distinct P450s in the metabolism and metabolic activation of B[*a*]P vs DMBA in mouse keratinocytes [7].

Luo et al. [24] revealed that furanocoumarins from the root of *A. dahurica* exhibited a significant inhibition on cell proliferation on several human tumor cells. Among the cells examined, imperatorin exhibited a marked inhibition of the growth of HepG2 cells. The results of various biochemical assays demonstrated that imperatorin triggered growth inhibition through apoptosis induction as evidenced by chromatin condensation and DNA fragmentation. The induction of apoptosis by imperatorin was found to be mediated through both death receptor and mitochondria pathways. Treatment with imperatorin resulted in significantly diminished expressions of procaspase- 3, pro-caspase-8, and pro-caspase-9.

Furthermore, the imperatorin -induced apoptosis was suppressed by caspase-8- and caspase-9-specific inhibitors and is believed to be mediated via both caspase-8 and caspase-9 [24].

Kim et al. (2006), by way of experiment with cultured human cells, showed the antiproliferative effect of furanocoumarins from *Angelica dahurica* (isoimperatorin, cnidicin, imperatorin, oxypeucedanin, byakagelicol, oxypeucedanin hydratate). The sulforhodamine B (*SRB*) assay was used in their work, and they adopted in the tumor cells, *in vitro* measuring by anti-cancer drug screening. This measured the inhibition range of cell proliferation after 48 hours, of cells that were treated with natural compounds isolated from *Angelica dahurica*. This study confirmed the cytotoxic activity of furanocoumarins, as well as imperatorin, by way of using five cultured human tumor cell lines. Depending on dose, the proliferation of tumor cells decreased significantly [18].

In a further study, in order to investigate the potential of *Glehnia littoralis* as a cancer chemopreventive, the antiproliferative effects of both its crude extracts and solvent-partitioned fractions (n-hexane, 85% aq. MeOH, n-BuOH, and water) were evaluated in HT-29 human colon cancer cells. The results of this work showed that its crude extracts and solvent-partitioned fractions exhibited dose-dependent inhibitory effects on cell proliferation. In particular, n-hexane and 85% aq. MeOH fractions especially exhibited a high antiproliferative effect, and induced apoptosis as determined by 4,6-diamidino-2-phenylindole (DAPI) staining, and reduced mRNA expression of Bcl-2, cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS). Systematic separation of n-hexane and 85% aq. MeOH fractions by diverse chromatographic methods led to the isolation of furanocoumarins (bergapten, isopimpinellin, xanthotoxin, imperatorin) and polyacetylene alcohols. All compounds exhibited dose dependent inhibitory effects on cell proliferation [46]. Other furanocoumarins (isoimperatorin, cnidicin, oxypeucedanin, byakagelicol and oxypeucedanin hydratate) were also revealed to have significant antiproliferative impact on tumor cells.

ANTIOXIDANT ACTIVITY

Free-radical-mediated cell injury and lipid peroxidation may be of critical importance in various pathological phenomena. Thus, antioxidants that prevent the damage caused by free radicals are considered to be worthy of study. It has also been suggested that structural specificity is involved in the manifestation of anti-oxidative activity of furanocoumarins. From research, the difference in the anti-oxidative activity of individual coumarin components on AAPH-induced cell damage is related to the position of their hydroxyl groups. Piao et al studied nine furanocoumarins which have no hydroxyl group in the aromatic ring and they showed little antioxidant activities. Hence, the hydroxy group in the aromatic ring is regarded as an important determinant for radical-scavenging and/or anti-oxidative potential. Further research has found that 9-hydroxy-4-ethoxypsoralen and alloisoimperatorin were the effective antioxidants among the various furanocoumarin components. This finding indicated that the aromatic hydroxy group plays a considerable anti-oxidative role by conferring stability to the radical form and participating in electron delocalization. Therefore, only some coumarin structures possess antioxidant activity [37].

ANTI-INFLAMMATORY ACTIVITY

Macrophages play a major role in host defence against infection and tumour development and this activity is regulated through the production of several mediators. Activation of macrophages causes the release of various cytokines, as well as chemicals such as nitric oxide (NO), prostaglandins and leukotrienes, which are important effector molecules that initiate inflammation. The oxidative metabolism of arachidonic acid leads to the synthesis of leukotrienes and prostaglandins through the cyclooxygenase and lipoxygenase pathway [14]. Because furanocoumarins are used in the treatment of skin lesions, as they produce very good results, they probably have some kind of anti-inflammatory activity.

Marques et al. isolated several furanocoumarins from the roots of *Oppopanax chironium* (L.), and evaluated them for their activity related to T-cell functionality. From the results of their research, heraclenin and imperatorin significantly inhibited T cell receptor-mediated proliferation in human primary T cells in a concentration-dependent manner. In transient transfection experiments with a plasmid containing the Interleukin-2, IL-2 promoter, they found that imperatorin is also a potent inhibitor of IL-2 gene transcription. Imperatorin seemingly inhibited both the nuclear factor of activated T-cells binding to DNA, as well as transcriptional activities, without significantly affecting the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells and the activator protein 1 transcription factors. These findings suggest the molecular mechanisms that are involved in the immunomodulatory and anti-inflammatory activities of imperatorun [28].

Abad et al. evaluated imperatorin as a potential inhibitor of some macrophage functions involved in the inflammatory process. By way of their research, imperatorin showed a significant effect on 5-lipoxygenase (leukotriene C4) and exhibited strong inhibition on cyclooxygenase-1- and cyclooxygenase-2-catalysed prostaglandin E2 release, with inhibition percentages similar to those of the reference drugs, indometacin and nimesulide. Their work showed that imperatorin caused a significant reduction of nitric oxide generation and can be classified as dual inhibitor, since it was evident that both cyclooxygenase and lipoxygenase pathways of arachidonate metabolism were inhibited by this compound [1].

ANTIBACTERIAL ACTIVITY

Shigella dysenteriae continues to be a major health problem which leads to death due to diarrhea and dysentery, predominantly in children below the age of 5. In this easily preventable disease, a bacterial invasion of the colonic epithelium leads to severe inflammation. This, together with bacterial dissemination generates abscesses and ulcerations. The periplasmic copper, zinc super oxide dismutase of *Shigella* protects it from the exogenous superoxide produced by the host during its invasion. Hence, an attempt was made to study the effect of an aqueous extract of *Aegle marmelos* on host and pathogen defence.

Histology analysis of rat ileal loop showed the loss of virulence in an aqueous extract of *A. marmelos* (200 mg/ml) pre-treated *Shigella,* and their intracellular survival was also decreased. The active component present in an aqueous extract of *A. marmelos* that brought this about, was identified as imperatorin. Evidence of its effectiveness was seen in the increase in peripheral blood mononuclear cell viability and the decrease in intracellular bacterial count as revealed by way of a transmission electron microscope analysis of imperatorin (50 mg/ml) treated *S. dysenteriae* that evidently had succumb to host oxidative stress. This loss of virulence is associated with attenuation of the copper, zinc super oxide dismutase activity in *Shigella*, which was confirmed by using the activity staining of bacterial cell lysate. Further, by performing docking analysis, it has been demonstrated that the imperatorin present in an aqueous extract of *A. marmelos* inhibited copper, zinc super oxide dismutase. From the above study, it was concluded that *Shigella* had succumbed to oxidative stress (host defence) due to inhibition of copper, zinc super oxide dismutase (the pathogen's defence mechanism) by imperatorin, an active compound aqueous extract of *A. marmelos* [40].

Smyth et. al. studied the antimicrobial activities of 43 naturally occurring (ie. imperatorin, among others) and synthetic coumarins against a range of *Gram-positive* and *Gram-negative* bacteria, including a hospital isolate of methicillin-resistant *Staphylococcus aureus* (MRSA). The coumarins that exhibited good bioactivity against two *S. aureus* strains were then assessed for their antimicrobial activities against a range of eight clinically isolated MRSA strains. Nearly one-half of the tested compounds displayed antimicrobial activity. Sixteen of these coumarins also possessed resistance-modifying activity. This reversed the resistance mechanism in MRSA, allowing the antimicrobial oxacillin to exert an enhanced effect against an MRSA hospital strain. When tested in combination with oxacillin, 8-iodo-5,7-dihydroxycoumarin had a similar activity to vancomycin, which is the current drug of choice for the treatment of MRSA infections [45].

It was found that imperatorin possesses an antilisterial activity with a MIC value more susceptible to *L. monocytogenes* ATCC 19116 (15.62 mg/mL) than *L. monocytogenes* ATCC 19111, 19166, 19118 and 15313 (31.25 – 62.5 mg/mL). The viable count assay showed that imperatorin at MIC concentration, strongly inhibited the growth of *L. monocytogenes* ATCC 19116. At a 60 min exposure of the imperatorin solution, a steep decline in colony-forming unit (cfu) numbers was observed against *L. monocytogenes* ATCC 19116. On scanning electron microscope observation, it was found that the treatment group showed partially collapse of cells or destruction in their morphology, swelling at endpoint with lysis of cell wall, partially distorted shape and pore formation. Thus, imperatorin had a detrimental effect on the morphological damages of *L. monocytogenes* [39].

ANTI-HIV-1 ACTIVITY

Coumarins and structurally related compounds have been recently shown to present anti-human immunodeficiency virus, type 1 (HIV-1) activity. Among these, the dietary furanocoumarin imperatorin imperatorin inhibits either vesicular stomatitis virus-pseudotyped or gp160-enveloped recombinant HIV-1 infection in several T cell lines and in HeLa cells. Imperatorin however, did not inhibit the reverse transcription nor the integration steps in the viral cell cycle. Using several 5-long terminal repeat-HIV-1 constructs, where critical response elements were either deleted or mutated, it was found that the transcription factor Sp1 is critical for the inhibitory activity of imperatorin induced by both phorbol 12-myristate 13-acetate and HIV-1 Tat. Moreover, in transient transfections, imperatorin specifically inhibited the phorbol 12-myristate 13-acetate-induced transcriptional activity of the Gal4-Sp1 fusion protein. Since Sp1 is also implicated in cell cycle progression, it was found that imperatorin strongly inhibited cyclin D1 expression and arrested the cells at the G1 phase of the cell cycle. These results highlight the potential of Sp1 transcription factor as a target for natural anti-HIV-1 compounds such as the furanocoumarins, which might have a potential therapeutic role in the management of AIDS [41].

HEPATOPROTECTIVE ACTIVITY

The number of patients with chronic hepatitis caused by hepatitis C virus infection is increasing world-wide. Chronic hepatitis caused by HCV infection finally develops into a hepatocellular carcinoma. In one study, the effects of imperatorin on Con A-induced liver injury was examined. In Con A-induced hepatitis, imperatorin had shown stronger effects than glycyrrhizin to decrease Con A-induced elevation of plasma alanine aminotransferase. It is postulated that a blockade of Ca2+ channels or phosphodiesterases might be involved in the protective effect of imperatorin on liver injury [31].

THE FUTURE PERSPECTIVES

Ethnomedicine has, for many years, revealed the positive biological activity of coumarins. Using modern methods of isolation and through specific rat and mice models, such as electroshocked induced seizures or chimney tests, a scien-

tific analysis of the pharmacological potential of various natural coumarin-containing components is allowed. Plants from the Apiace and Rutaceae families are the basic sources for these active compounds, however, most of the coumarins are available in dietary vegetables or in candies, alcohol beverages and fragrances. This data should be also taken into consideration during the assessment of their future perspectives as pharmaceutical agents.

CONCLUSIONS

In such demanding expectations, the coumarins with their various, wide biological activity seem to be *the panacea* for many diseases. In this regard, imperatorin, being a very widely examined coumarin can be an important perspective for future therapy enhancement. By way of research, it is already known that its neuro-activity on GABA level is crucial in improving the treatment of the neurodegenerative diseases as epilepsy, Parkinson disease, neuropathic pain, as well as other neuro-diseases. Imperatorin can also influence conventional anti-epileptic treatment by way of various mechanisms such as decreasing the ED_{50} of several drugs used in treating this neurodegenerative disorder. In addition, it has been shown that it can modify the permeability of the blood/brain barrier for anti-epileptic drugs such as carbamazepine. The other recognized imperatorin function is in its positive activity on blood pressure, as compare with verapamil, by way of its ability to block the calcium channel, as well as its action on myocardial hypertrophy. In several studies, imperatorin also explicitly suppressed the increase of the size of the cells induced by angiotensin II, which seems to be the next positive prognosis for the treatment of hypertension. As to cancer, the antiproliferative activity of coumarins could be important for tumor treatment, hence this is worthy of investigation.

In this review, the role of imperatorine in drug therapy was presented (based on several studies), in comparison to other coumarins, so as to show its rank in the pharmacological activity within the coumarin group. This review also pointed out that imperatorin, as with the other coumarins, has a very wide range of additional effects beyond that mentioned within this article, including anti-inflammatory and antimicrobial activity. This too is worthy of further investigation.

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