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Review Article

Anticancer Properties and Clinical Trials of Coumarins: A Review

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ABSTRACT
Cancer has evolved as one of the most common causes of mortality, worldwide. Though numerous chemotherapeutic treatments are available, their side effects such as cytotoxicity and drug resistance form a big problem during the cancer treatment. Recent studies of anticancer activities conducted on natural products isolated from plants, namely coumarin and related compounds, prove them to be a promising drug candidate in cancer treatments. Efforts made by the scientists to design and develop novel anti-cancer agents using coumarins as lead compounds and study their effectiveness using Structural Activity Relationship is worth appreciating. This review, therefore, focuses on the recent progress in the discovery of coumarin derivatives with potential antitumor activity. It also summarizes their structure-activity relationship, and mechanism of action studies.
Keywords: Cancer, Cytotoxicity, Antitumor activity, Coumarins, Natural produtcs, Structural Activity Relationship.

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INTRODUCTION

Cancer is an uncontrolled growth of abnormal cells in the body causing rapid DNA replication and inhibition of protein secretion of normal cells.[1] The rate of this deadly disease is highest in economically developing countries due to highly prevailing factors like smoking, unhealthy diet, physical inactivity, and birth controls. In 2020, the International Agency for Research on Cancer (IARC) estimated that there were 19.3 million new cancer cases and 10.0 million cancer deaths worldwide. Among the new cases, 10.1 million cases were in men and 9.2 million in women. Breast and lung cancers were the most common, contributing 11.7% and 11.4% of the total number of cases. By 2040, cancer cases are expected to grow to 28.4 million new cases.[2] Regardless of great progress in the field of Oncology; many types of cancers, unfortunately, remain cureless. Conventional treatment therapies are hopeful to a significant extent, but at the cost of normal cells. Therefore, cancer research currently aims to target critical molecules that will treat the disease as well as avoid damage to the body. Such newly discovered or synthesized drugs should have tumor selectivity, efficiency, and safety principles together with the minor environmental impact on clinical applications.[3]

NPs, when compared to the conventional synthetic molecules, are characterized with complex structures and enormous scaffold diversity with promising bioactivities. Their structural analogues have historically made a major contribution to pharmacotherapy, especially for cancer and infectious diseases.[4] Coumarins are multi-targeting heterocyclic organic compounds belonging to the class of benzopyrones[5] that are widely distributed in nature.[6] They have been also reported as effective chemopreventive and anticancer agents *in vitro* and *in vivo*.[7] Coumarin derivatives such as Irosustat, can exert diverse antiproliferative mechanisms and is under clinical trials.[8] Flavonoids are polyphenolic compounds synthesized by plants that possess anticancer activities such as arresting the cell cycle, inducing apoptosis, autophagy, and suppressing cancer cell proliferation and invasiveness.[9] These NPs recently gained importance as anti-cancer agents with cytotoxicity potential promoting apoptosis in cancer cells.[10]

Therefore, it is necessary to study the anticancer property of these natural products in detail. In this review, a summary of beneficial properties of coumarins, flavonoids and benzopyrones have been discussed along with their effective mechanisms in treating cancer.

Coumarin, derived from a French term "Coumarou", was isolated for the first time from 'Tonka beans' in 1820 by Vogel.[11] This large class of naturally occurring phenolic compounds are classified as simple coumarins (e.g. coumarin), linear furanocoumarins (e.g. isopimpinellin), angular furanocoumarins (e.g. angelicin), linear pyranocoumarins (e.g. xanthyletin), angular pyranocoumarins (e.g. seselin),[12,13] dihydrofurano coumarins (e.g. marmesin), phenyl coumarins (e.g. disparinol D) and bicoumarins (e.g. dicoumarol).[14]

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FURANOCOUMARINS

Due to their numerous clinical applications, furanocoumarins are a therapeutically significant subgroup. The 5-membered furan ring that is connected to the coumarin nucleus makes up the furanocoumarins. Psoralen (Linear) and angelicin are two of the most significant and popular furanocoumarins (Angular). The furan ring's relationship to the coumarin nucleus is indicated by the phrases linear and angular.[15] Psoralens are naturally occurring plant biosynthetic metabolites that have been utilised in phototherapy to treat a variety of skin conditions, such as mycosis fungoides, psoriasis, and vitiligo, since ancient times.[16] In recent years, psoralens have been used in concert with anti-sense technology to control the proliferation of human cervical cancer cells.[17] By repressing the gene expression in a sequence-specific manner, oligonucleotides and their analogues have been utilised to prevent protein production. The approach, known as the antisense strategy, has been used in gene therapy for terminal illnesses like cancer and viral infections. Numerous papers on antisense technology have provided convincing evidence that the antisense mechanism played a role in controlling cell proliferation. S-interactions oligo's with certain proteins, such as growth factors, may be responsible for some of the antiproliferative effects of oligo nucleoside phosphorothioates. Psoralen derivatives have the capacity to covalently crosslink with pyrimidine bases when exposed to UVA radiation (e.g. thymine and uracil). Psoralen derivatives have been conjugated with oligonucleotides to increase the antisense effects since they can cross-link genes to inactivate their expression. Psoralen-conjugated S-oligos have demonstrated considerable inhibitory effects to UVA radiation in *in vitro* tests due to their resistance to nucleases. Human cervical cancer cell growth was stopped using psoralen conjugated S-oligos (Ps-S-oligo). Human cervical cancer cells that have been exposed to Ps-S-oligo greatly reduced proliferation after UVA radiation. Ps-S-oligo is complementary to the initiation codon region (Ps-P-As) of the human papillomavirus (HPV)18-E6*-mRNA. The inhibition of the E6* protein may control cellular proliferation since it is closely associated with the metamorphosis of human cervical cells. The psoralen-conjugated antisense DNA has a large ability to control gene expression, which may provide important information to investigate novel genes regulating agents.[17]

PYRANOCOUMARINS

Plant products have a long history of being utilised to treat cancer, both as chemotherapeutic drugs and as complementary therapies. Radix peucedani, a plant used for treating pulmonary hypertension and respiratory illnesses, yielded the pyranocoumarin complex (+)-3-angeloyl-4-acetoxy-cis-khel-lactone. One of the biggest challenges to developing a cancer treatment that works is the resistance of cancer cells to chemotherapeutic drugs. Multidrug resistance (MDR) in cancer cells may be caused by over-expression of membrane drug efflux pumps, p53 mutations,[18] up-regulation of Bcl-2,[19] DNA repair,[20] or cellular detoxification enzymes.[21] P-glycoprotein is overexpressed in some MDR cell lines and works as an ATP-dependent drug efflux pump to rapidly expel anti-tumor medicines from target cancer cells, preventing the medications from having their deadly effects.[22] (+) Studies have recently been studied to determine the effects of P-glycoprotein inhibitor -3-angeloyl-4-acetoxycis-khel-lactone on MDR cell lines. Apoptotic cell death is induced by this pyranocoumarin in drug-sensitive KB-3-1 and multidrug resistant KB-V1 cancer cell lines, according to research by Wu[23] and colleagues. There were pronounced synergistic interactions when pyranocoumarin was used with well-known anticancer drugs as vincristine, doxorubicin, and paclitaxel. As an MDR reversal agent, this pyranocoumarin may be useful in medicine. But additional research is need to verify these latest findings.

WARFARIN

One stage of metastatic process involves the tumour cell entering the bloodstream and stopping in a capillary at a distant region where a metastatic tumour would eventually develop. By trapping cells in capillaries or by making it easier for cells to attach to capillary walls, blood-clotting pathway components may help metastasis. Anticoagulants could theoretically obstruct this stage of the metastatic process.[24] In the therapy of SCCL (Small Cell Carcinoma Lung), a tumour cell type that is distinguished by a coagulation-associated pathway, warfarin has demonstrated particularly encouraging outcomes.[25-27] Research by Mousa[28] has shown that anticoagulation with commonly prescribed drugs like warfarin (Coumadin) and unfractionated heparin inhibits tumour growth by lowering the ability of tumour cells to be retained in the pulmonary microvasculature. In patients with malignancies, recent research suggests that cimetidine therapy combined with anticoagulant drugs may improve cancer survival and reduce metastasis. The adhesion of the highly invasive breast cancer cell lines BT-549 and MDA-MB-231 was investigated and studied in a study using cimetidine, anticoagulants, and a combination of cimetidine and anticoagulants. There were notable anti-adhesion effects of both warfarin and cimetidine. It is possible to lessen tumour angiogenesis and adherence with warfarin and other anticoagulants. Cimetidine and anticoagulant medicines have an enhanced anti-adhesion impact and other anti-metastatic properties.[29] The biology of malignant tumours is now strongly supported by a large body of evidence that the blood coagulation system is important. This information was derived from a variety of clinical, biochemical, histological, and pharmacological research, and it suggests that drugs that interfere with blood coagulation pathways may be effective in affecting the course of malignant disease.[28]

PHARMACOLOGICAL PROPERTIES OF COUMARINS

High Protein Oedema (HPO)

The lymphatic system is in charge of removing interstitial fluid from within human tissues. Edema hinders tissue cells' ability to function and limits oxygen transfer, which makes it difficult for wounds to heal.[30] In the case of high protein edemas (HPO), there is an accumulation of protein in the tissue as a result of injury or inflammation, and this causes the capillaries to become more permeable, allowing water to leak into the tissue spaces. High protein edemas are linked to a wide variety of diseases, from the exceedingly severe and chronic (such lymphoedema and elephantiasis) to the more prevalent and acute types (e.g. burns, accidental and surgical traumas). Treatment with benzopyrone has been demonstrated to be beneficial for all kinds.[31] In studies on high protein oedema, coumarin and various other benzopyrones have all been found to effectively reduce swelling. However, Loprinzi[32] and colleagues assert that coumarin therapy alone is ineffective for women who have arm lymphoedema following breast cancer treatment. By employing coumarin in combination therapies with other substances, it would be able to enhance the good therapeutic effect of this substance. The aim of a recent study was to assess the edema-protective impact of coumarin/troxerutin (SB-LOT) plus compression stockings in patients with chronic venous insufficiency following leg decongestion as advised by the current recommendations. According to the study, SB-LOT has an edema-protective effect in chronic venous insufficiency and offers patients who stop wearing compression after a short period of time a therapeutic alternative.[33]

Chronic Infections

In line with current recommendations, a new study sought to determine if compression stockings and coumarin/troxerutin (SB-LOT) had any edema-protective effects in patients with chronic venous insufficiency after leg decongestion. The study found that SB-LOT has an edema-protective effect in chronic venous insufficiency and provides patients who discontinue wearing compression after a short while with a therapeutic alternative.[33] In chronic brucellosis, infected macrophages by *Brucella abortis* elude immune response.[34] Symptoms of this chronic brucellosis disappear after the administration of immunostimulatory drugs such as coumarins. This encouraged the use of coumarins against other chronic infections like mononucleosis, mycoplasmosis, toxoplasmosis and Q fever. A new antiplasmodial coumarin has been isolated from the roots of *Toddalia asiatica*. This finding supports the traditional use of this plant for the treatment of malaria.[35]

Other pharmacological effects

In addition to their herbicidal and insecticidal activities, furocoumarins have antibacterial, fungal, insecticidal, larvicidal, moluscicidal, nematicidal, and ovicidal capabilities.

POTENTIAL APPLICATION OF COUMARINS IN CANCER

The primary goal of conventional anti-cancer drugs is to stop normal cell division in order to harm the abnormally dividing cell.[36] Topoisomerase inhibitors, DNA cross-linking agents, agents that disrupt the cytoskeleton, and antimetabolites are among the reagents used. Vinblastin, adriamycin, and campothecins are some examples of these treatments (e.g. mercaptopurine). Despite these drugs' efficiency, the haematopoietic system and other typically reproducing tissues are particularly susceptible to their severe side effects. Combination therapies, in which different cytotoxic medications are used in the treatment routine, typically offer superior outcomes with fewer negative side effects because they are carefully managed to allow recovery of normal, but not malignant cells after drug exposure.[36] For cancer patients, the best outcomes currently come from combining surgery, radiation, and chemotherapy. Certain cancer types, including Hodgkin's lymphoma, testicular cancer, and various leukemias, have been successfully treated with certain therapy regimens. Coumarins can not only treat cancer but also lessen the side effects of radiotherapy. Recently, the efficacy of coumarin/troxerutine combination therapy for the preservation of salivary glands and mucosa in patients undergoing head and neck radiation therapy was investigated. The results demonstrate that radiogenic sialadentis and mucositis can be effectively treated with coumarin/troxerutine.[37] There has been interest in coumarin and 7-hydroxycoumarin as potential anti-cancer medications due to reports that these substances have had objective responses in some patients with advanced malignancies.

Coumarin in Malignant Melanoma

If malignant melanoma is found early enough, it can be surgically removed and has a favourable prognosis. Since there is now no effective treatment for recurrent malignant melanoma, the chance of recurrence rises if the lesion progresses and presents the oncologist with major difficulties. As per studies, five years post the original lesion, malignant melanoma recurrence occurred in 55–80 percent of high-risk patients.[38] In the initial studies using coumarin derivatives to treat melanoma, warfarin played a crucial role. This substance has been demonstrated to stimulate and enlarge macrophages, lymphocytes, and granulocytes.[39] Thornes then started to consider whether the forerunner of warfarin, coumarin, could be used as an adjuvant treatment for melanoma. The *in vivo* effects of coumarin were previously thought to be caused by macrophages, similar to how warfarin works. Prior administration of coumarin was non-toxic, easy to use, and had no anti-coagulant impact, all of which led to a subjective improvement in the health of cancer patients.[40,41] In a more recent study, Velasco-Velazquez and colleagues[42] examined the effects of 4-hydroxycoumarin on murine melanoma cell line B16-F10 and non-malignant fibroblastic cell line B82 (4-HC). B16-F10 cells but not B82 fibroblasts showed altered actin cytoskeleton after exposure to 4-HC.

Coumarin in Renal Cell Carcinoma

Renal cell carcinoma (RCC) has a well-known clinical history, and its hallmarks include rapid tumour growth and extended stable intervals. Surgery remains the preferred course of action for patients whose tumours are kidney-specific. But many of these patients quickly develop recurrent or metastatic disease, with the lungs, liver, and bones being the most frequent secondary sites of recurrence.[38] It is a grim prognosis because less than 10% of people with metastatic RCC will live for 5 years.[43] The coumarin class of compounds attracted attention after Thornes' studies on their immunomodulatory properties and use in malignant melanoma.[44] The therapeutic activity of coumarin in patients with renal cell carcinoma has been investigated using the Thornes therapy regimen (coumarin (1,2-benzopyrone), the parent compound of warfarin, at 100 mg/day orally with addition of cimetidine 4 × 300 mg/day starting on day 15). This exploratory trial provided some fascinating results with 45 patients with metastatic RCC, 14 objective results, and almost no adverse effects. This anti-tumor impact has also been demonstrated by other researchers.[45,46] Following this success, it was clear that additional knowledge regarding dosages and toxicities was required, thus Marshall and colleagues carried out a phase I trial to identify the maximum tolerated dose and dose-limiting toxicities of coumarin and cimetidine. Across all coumarin doses, the most prevalent side effect, nausea, was well tolerated, probably as a result of the coumarin's strong smell. Across a range of coumarin dosages (600–5000 mg), seven patients with renal cell carcinoma all demonstrated objective responses.[47] Recent research has investigated the *in vitro* cytotoxic potential and mechanism of action of certain coumarins employing renal cell lines.[48] The results suggest a potential therapeutic role for the coumarins under study in the treatment of renal cell cancer.

Coumarin in Prostate Cancer

The most common invasive cancer in males, prostate cancer has a very slow growth rate and a wide spectrum of biological variability, particularly in terms of hormone sensitivity.[49] These two traits have restricted attempts at curative therapies for patients because the majority of efficient chemotherapeutic drugs rely on rapid growth kinetics in the tumour mass and hormonal therapy (androgen limitation) is not always effective because of differential hormonal dependence. The present focus of clinical approaches is the early detection and surgical or radiation removal of clinically relevant cancers. Local lymph nodes and bone are the primary metastatic locations, and metastases are what determine whether a patient survives. Almost all prostate tumours that at first responded to androgen restriction will eventually revert to a hormonally insensitive status, and expand in the absence of testosterone. It is obvious that better therapeutic approaches are required to control both metastases and hormone-insensitive prostate cancer. As it had previously been demonstrated that coumarin had immunomodulating effects on other malignancies, the efficiency of coumarin in treating prostate cancer was examined in a small-scale trial.[50] Using 40 patients with metastatic, hormone-naive, or hormone-refractory prostatic cancer, a phase I trial was conducted.[51] Participants received daily oral administration of 3g of coumarin to ensure compliance. Monthly assessments of toxicity and

anti-tumor effects were made. Partially responsive patients included three people with low tumour loads. One responder continued to have three responsive bone metastases after the trial while maintaining steady PSA levels for seven years. Myers[52] and colleagues investigated the effects of coumarin on the growth of two cell lines for malignant prostatic cancer (0-500 mg/ml) and two cell lines for renal cell carcinoma (786-O and A-498) (DU145 and LNCaP). After 5 days of treatment, coumarin slowed down the growth of the four cell lines. The LNCaP prostatic cell line was particularly susceptible to the inhibitory effects of coumarin.

Coumarin in Leukaemia

Researchers have looked into how esculetin, coumarin, and 7-hydroxycoumarin affect the cell cycle and the chemicals that control it. Using *in vitro*-grown human and animal cell lines, it was examined whether 8-nitro-7-hydroxycoumarin (8-NO_2-7-OHC) had cytostatic or cytotoxic properties. In two examined human cell lines (HL-60 and K562), this substance exhibited cytotoxic characteristics that caused cell death by apoptosis. By interfering with their cell cycle, this substance had a cytostatic effect on the other three cell lines that were evaluated.[53] Cooke[54] looked at how several coumarin compounds affected the development, metabolism, and cell signalling of human tumour cell lines. On the evaluated cancer cell lines, esculetin had the strongest overall antiproliferative effects.[55] Testing was done to see if the cellular target of 7-hydroxycoumarin was a part of the signalling pathway due to the significance of signalling aberrations in cancer cells. The tyrosine phosphorylation of EGF-stimulated tumour cells was discovered to be inhibited by 7-hydroxycoumarin and esculetin in a time- and dose-dependent manner. It appears that this impact may be accomplished by lowering the EGF-tyrosine Receptor's kinase activity.[54] Evidence from a recent study suggests that esculetin impacted pRB's phosphorylation, causing G1 arrest in human leukaemia HL-60 cells. The outcomes showed that esculetin administration led to a buildup of hypophosphorylated pRB in HL-60 cells as well as a decrease in both cyclin D1 and E. The cell cycle was induced to stop in the G1 phase as a result. The E2F family of transcription factors is prominent among the released proteins. E2F not only promotes DNA synthesis-related gene expression, but it also helps control the cyclin D1 and E genes. Esculetin administration also resulted in increased CDKI p27 expression and decreased CDK-4 expression, which prevented pRB phosphorylation.[56] In a different study, the impact on cell cycle progression of the human adenocarcinoma cell line A427 was examined in order to understand the mechanisms of action of 7-hydroxycoumarin and coumarin. These cells exhibit homozygous deletions at the p16INK4A gene and are pRB-positive. According to the findings, coumarin and 7-hydroxycoumarin both had higher cytostatic potency. The cytostatic impact of 7-hydroxycoumarin is consistent with the suppression of the cell cycle at transition G1/S. Additionally, the decline in the proportion of cells expressing cyclic D1 shows that early G1 phase events are involved in 7-hydroxycoumarin's activity. Lack of cyclin D1 mRNA level alterations points to a post-transcriptional action of 7-hydroxycoumarin. If this pathway is blocked, GSK-3 phosphorylation, which results in cyclin breakdown, cannot be prevented.[57]

Coumarin in breast cancer

The clinical uses of benzopyrones have been the subject of a sizable amount of research, however it is crucial to stress that only the "warfarin-type" anticoagulants fall under the purview of mainstream pharmacotherapy. Other coumarin compound varieties are either in early clinical trial stages or are still in the experimental stage. To assist a better understanding of these chemicals and enable potential clinical applications, additional research on the elucidation of their mechanism of action is required. Cell proliferation-based methodologies were used in earlier studies of coumarin chemosensitivity.[52,54] A new selective oestrogen receptor modulator (SERM), raloxifene, and tamoxifene were recently tested for their ability to inhibit tumour growth in both an *in vitro* and *in vivo* MCF-7 breast cancer model using an *in vitro* proliferation assay.[58] Warfarin, 7-hydroxycoumarin, esculetin, and genistein are four benzopyrones that have recently been studied for their impact on the growth and metabolism of two cell lines, MCF-7, a breast cancer, and A549, a lung carcinoma. To further understand the ways that genistein, esculetin, and warfarin work, signal transduction studies on MCF-7 cells were also carried out. As a result, genistein's ability to prevent growth was more effective than esculetin, which was better than warfarin. All three substances had a very strong effect on MCF-7 cells. Compared to MCF-7 cells, A549 cells did not appear to be as susceptible to the effects of all the tested substances. On A549 cells, however, genistein and esculetin both showed strong inhibitory effects. The sensitivity of both cell lines examined to esculetin's growth inhibition was the most obvious pattern from the results (6,7-dihydroxycoumarin). This is consistent with earlier findings by Cooke and O'Kennedy[55] and Kolodziej[59] and colleagues that a dihydroxy-function in either an ortho- or meta-format was a very effective chemical structure for toxicity in human tumour cell lines. A double hydroxy-function on the coumarin ring may be the cause of the increased potency because this potency was not present in any of the single hydroxycoumarin compounds.[60] Endpoints for cytotoxicity testing frequently include evaluation of a wide range of enzyme leaks. The widespread consensus that coumarin is not the cause of the reported *in vivo* effects but rather a pro-drug for other active metabolites appears to be confirmed by the insensitivity of a number of human tumour cell lines to the growth inhibition by coumarin.

PHARMACOKINETICS

Over many years, the pharmacokinetics of coumarin, including the excretion of several metabolites, were clarified. With little excreted in the form of an unaltered molecule, coumarin is swiftly and nearly fully metabolised.[61]

Absorption and Distribution

The body quickly absorbs coumarin after oral administration and distributes it throughout.[62] Water does not readily dissolve coumarin or 7-hydroxycoumarin (0.22 and 0.031 percent, respectively). Since 0.3 percent solubility in water is thought to be the key number at which the dispersion of a compound restricts its rate of absorption, these percentages suggest that coumarin may have reduced bioavailability *in vivo*. However, both substances have high partition coefficients (21.5 percent for coumarin and 10.4 percent for 7-hydroxycoumarin), which is thought to be advantageous for the quick absorption of substances once they are in aqueous solution. This, along with the fact that coumarin is non-polar, means that, in theory, coumarin should be able to passively diffuse through lipid bilayers.[54] Following oral treatment, coumarin is entirely absorbed from the GIT and severely degraded by the liver in the first pass, with just 2 to 6 percent of it making it to the systemic circulation intact.[62] It is now recognised that coumarin is a pro-drug, with 7-hydroxycoumarin being the chemical of primary therapeutic relevance due to coumarin's low bioavailability and short half-life (1.02 hr orally vs. 0.8 hr intravenously). Many medicines are present in the plasma mostly in the bound form at typical therapeutic plasma concentrations. Thirty-five percent of coumarin and forty-seven percent of 7-hydroxycoumarin bind plasma proteins, according to research by Ritschel and colleagues.[63] Since the proportions that bound were significantly below the established threshold value of 80% binding, availability of the chemicals to their target tissues shouldn't be a concern. Numerous species, including the rat, dog, gerbil, rhesus monkey, and man, have had their pharmacokinetics for coumarin examined.[62] Very

specialised and sensitive analytical techniques are based on the specific antibody recognition of its antigen. This was used in many different ways to determine the pharmacokinetics of coumarin and its derivatives. For the detection of coumarin and 7-hydroxycoumarin in urine, immunoanalytical techniques have included ELISA-based techniques.[64] Additionally, surface Plasmon resonance (BIAcore) or electrochemistry-based biosensors based on antibodies have been used to detect coumarin chemicals in diverse matrices.[38,65]

Metabolism

The metabolic destiny of coumarin has been extensively studied because, historically, pharmacologists have seen it as the ideal model for studying the complex metabolism of an organic molecule with a simple structural makeup.[62,66,67] The metabolic destiny of coumarin must be determined in order to take advantage of the fact that it is metabolised at several sites and to access any potential metabolic dependence of coumarin-induced toxicity.[61] In the beginning, coumarin is converted to 7-hydroxycoumarin by the particular cytochrome P-450-linked mono-oxygenase enzyme (CYP2A6) system in liver microsomes. Coumarin undergoes a phase II conjugation reaction after being 7-hydroxylated, producing a glucuronide conjugation that is connected to 7-hydroxycoumarin. When compared to the livers of other animal species, the 7-hydroxylase activity in human liver microsomes is remarkably high. While nonexistent in human microsomes, coumarin 3-hydroxylase activity is quite high in rodent microsomes. Although hydroxylation of coumarin can occur at any of the six potential positions (i.e., carbon atoms 3, 4, 5, 6, 7, and 8), the most frequent sites produce 7-hydroxycoumarin and 3-hydroxycoumarin, respectively (Figure 1). Among the different metabolic processes, 7-hydroxylation has drawn the greatest interest, mainly because it is the main human metabolic pathway and is simple to study. O-hydroxyphenyllactic acid (o-HPLA) and o-hydroxyphenylacetic acid (o-HPAA) are two additional products produced by hydroxylation at carbon 3 as a result of further metabolism via ring opening.[61,62] Due to genetic and environmental influences, each person expresses CYP enzymes (like CYP2A6) differently. Inter-individual variance in the metabolism of medications like coumarin is caused by these factors. Different species, races, and ethnic groupings exhibit poor metabolizers more frequently than others. Large inter-species and intra-individual heterogeneity in the activity of these enzymes has been demonstrated.[68]

In vivo and *in vitro* studies on the metabolism of coumarin have been conducted in a variety of animals, including humans. Oral dosing followed by urine collection with or without scheduled fractionation is typically used in human metabolic research.[69,70] A variety of methods are used for analysis, including capillary electrophoresis, HPLC, and spectrofluorimetry.[71,72] Hepatocytes, subcellular fractions, pure and cDNA-expressed enzymes, tissue slices, and other *in vitro* systems have all recently been used.[62] The majority of human study subjects undergo substantial 7-hydroxycoumarin metabolization of coumarin. Table 1 displays some information for various coumarin dosage levels and collection times. Following an oral dose of coumarin, the measurement of urine 7-hydroxycoumarin has been used as a biomarker of human hepatic CYP2A6, the cytochrome P-450 (CYP) isoform that is in charge of 7-hydroxylation of coumarin in human liver.[61] Some people can metabolise coumarin in significant amounts through processes other than 7-hydroxylation, like the 3,4-epoxidation pathway to o-HPAA. The CYP2A subfamily in humans consists of three genes, although CYP2A6 is primarily more significant because the two other gene products, CYP2A7 and CYP2A13, are either inactive or do not express in the liver. About 10% of all P450 enzymes are encoded by the CYP2A6 gene.[73] This enzyme catalyses the coumarin 7-hydroxylation. It has recently been discovered that the human liver expresses CYP2A6 polymorphically. It has been established that CYP2A6 takes involvement in the metabolism of nicotine and its metabolite, cotinine. This enzyme is also involved in the metabolism of some medicines and substances, including coumarin, which is frequently employed as a probe material for CYP2A6 both *in vitro* and *in vivo*.[74] Pelkonen[73] has compiled a list of substances and inhibitors that are currently understood to be metabolised by or interfere with CYP2A6 *in vitro* and *in vivo*. Although 7-hydroxycoumarin is the primary human metabolite, additional hydroxylation mechanisms are significant in humans and, as a result, the therapeutic importance of non-7-hydroxymetabolites should be investigated rather than ignored.

Table 1: Extent of coumarin metabolism to 7-HC in various species. [a]coumarin administered orally unless otherwise stated.[75,76]

Species	Dose (mg/kg)[a]	Collection time (hr)	Urinary 7-HC (% of Dose)
Rat	100	890 or 120	0.4
Mouse	21. i.p.	22	25
Syrian Hamster	200	24	5
Squirrel Monkey	200	24	1
Baboon	200	24	60
Human	200mg/subject	24	[b]79 (range 68-92)
	200mg/subject	24	[c]63 (range 40-97)

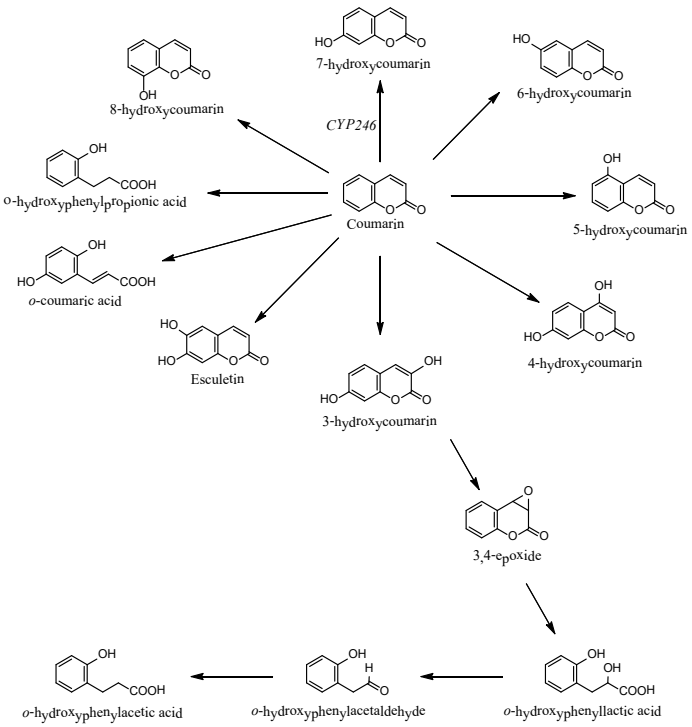

Figure 1: Metabolism of coumarin. All biotransformation are possible, although the metabolism is species-specific.

Isoflavones

Researchers from the fields of medicine, pharmacology, and plant physiology have been studying isoflavones for many years. The biological activity of isoflavones is influenced by where the phenyl ring is located in relation to the third carbon of the benzo-δ-pyrone. Isoflavones have incredibly intriguing, multidirectional medicinal properties. This

means that these substances have anti-inflammatory, antimitotic, and radical-scavenging capabilities in addition to both estrogenic and anti-estrogenic activities.[77] A natural phytoestrogen isoflavone found in soy is called genistein (4, 5, 7-trihydroxyisoflavone). People who consume large amounts of soy in their diets produce more free genistein and other related isoflavones in the gastrointestinal tract, which are present in the circulating blood, build up in tissue, and are eliminated in the urine. According to studies, those who eat a traditional diet heavy in soy products have a lower incidence of some cancers, such as breast, prostate, and colon cancer.[78] Because genistein is a well-known tyrosine kinase inhibitor, it has been demonstrated that it inhibits the proliferation of cancer cells *in vitro*. It was decided to test this substance's impact on two cell lines *in vitro*. Background information on this crucial substance for medicine is provided in the section that follows.

Genistein

Although genistein is a benzopyrone, it is not considered a coumarin (benzo-a-pyrone). A member of the benzo-g-pyrone family, it is a flavonoid. A member of the "isoflavone" class of substances, genistein is a phytoestrogen. These diphenolic substances physically mimic estradiol (E2) and have been found to have some estrogenic action.[79] In the past few years, there have been numerous reports of environmental estrogens, also known as xenoestrogens. There are significant differences between estrogenic chemicals of an industrial origin and those with a plant origin. Some experts believe that substances like the industrial PCBs and the pesticide DDT can lead to populations exposed to them developing malignancies that are oestrogen dependent. Phytoestrogens are quickly digested and remain in the body for a short period of time, in contrast to some industrial xenoestrogens that have a tendency to bioaccumulate in adipose tissue and linger there for years. It's vital to consider the time, frequency, and intensity of phytoestrogen exposure. Isoflavones and lignans are the two main types of phytoestrogens that have drawn the greatest interest from scientists. One of the key factors contributing to the much-reduced incidence of breast cancer in China, Japan, and South East Asia has been suggested to be diets high in naturally occurring anti-estrogenic compounds such isoflavones. Isoflavones like genistein and daidzein are particularly common in soybeans.[80]

Genistein in Cancer Research

In vitro tests have revealed that genistein inhibits the multiplication of cancer cells. The cause of this action is thought to be the suppression of numerous important enzymes, particularly tyrosine kinase, which is essential for cell growth and transformation. Tyrosine kinase is also connected to the production of oncogenes in breast cancer. One of the most often diagnosed cancers in women is breast cancer, and incidence rates are rising in developed countries. Genistein's impact on human dysplastic and malignant epithelial breast cell lines that display low and high metastatic potentials has been studied by Tanos[79] and colleagues. They found that genistein significantly slows the growth of dysplastic (fibrocystic cells) and malignant breast cells *in vitro*. When cancer cells were exposed to a genistein and tamoxifen therapy, *in vitro* tests revealed that genistein also had a synergistic additive impact. These findings point to the potential of genistein administration either alone or in conjunction with tamoxifen for the treatment of breast cancer.[79] On g-irradiated K562 myeloid leukaemia cells, genistein therapy causes cell cycle arrest and has a role in cell death, according to a different study.[80] Genistein shares structural similarities with estradiol (E2) and has been shown to have mild estrogenic activity in various investigations.[78,81,82] Depending on the levels of circulating endogenous estrogens and oestrogen receptors in humans, this substance may have both estrogenic and antiestrogenic effects (ER). The protective effects of genistein against atherosclerosis, coronary artery disease, osteoporosis, and post-menopausal manifestations of hot flushes and other symptoms may be due to its oestrogen agonistic activity in the absence of oestrogen. Similar pathways may also be responsible for genistein's preventive effects against several hormone-dependent malignancies, including prostate cancer.[82]

CONCLUSION

Previous investigations on coumarin and its derivatives have been clinically proven to treat deadly cancers such as leukaemia, prostate cancer, renal cell carcinoma, melanoma, breast cancer, lung carcinoma, etc. Due to their high biopotent nature, coumarins are capable of treating various other disorders including heart failure and hyperglycaemia. Research is still required on a very large scale wherein the synthesis of coumarin derivatives with various functional groups can be achieved, that will reduce its existing hepatotoxicity side effects. Coumarins and related compounds, both synthetic and natural, are a promising anticancer lead compounds that will result in new drug discoveries.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

Authors' Contributions

All authors made a substantial contribution to this work. **GE-SB:** Conceptualization, Methodology, Validation. **JNN:** Conceptualization, Investigation, Data curation, Writing - review and editing. **NS, JG, VRP:** Investigation, Data curation, Writing- Original draft preparation. All the authors read and approved the final manuscript.

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