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Review

Anticancer and Anti-Inflammatory Effects of Tomentosin: Cellular and Molecular Mechanisms

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Abstract: Tomentosin is a natural compound known for its presence in some medicinal plants of the Asteraceae family such as *Inula viscosa*. Recent studies have highlighted its anticancer and anti-inflammatory properties. Its anticancer mechanisms are unique and act at different levels ranging from cellular organization to molecular transcriptional factors and epigenetic modifications. Tomentosin's possession of the modulatory effect on telomerase expression on tumor cell lines has captured the interest of researchers and spurred a more robust study on its anticancer effect. Since inflammation has a close link with cancer disease, this natural compound appears to be a potential cancer-fighting drug. Indeed, its recently demonstrated anti-inflammatory action can be considered as a starting point for its evaluation as an anticancer chemo-preventive agent

Keywords: tomentosin; cancer; inflammation; telomerase; apoptosis; NF-κB

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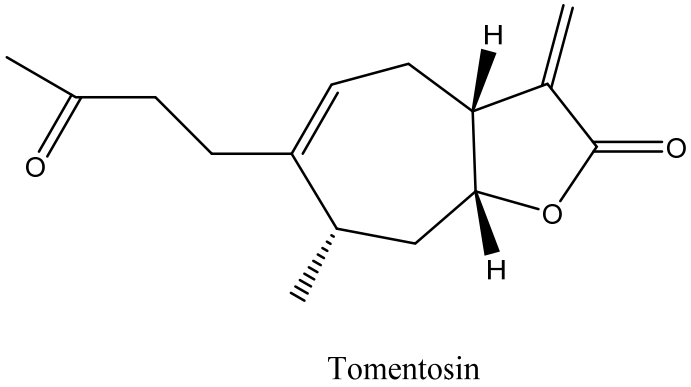
1. Introduction

Cancer is a complex and multifactorial disease in which several risk factors are interconnected and can lead to tumor transformation. These risk factors can affect several levels including genetic, biochemical, and epigenetic mechanisms [1,2]. These mechanisms can directly or inter-directly cause or initiate the transformation program of a normal cell into a cancerous cell. Moreover, other risk factors such as infectious diseases, tissue or cell damages can also, directly or indirectly, induce a chronic inflammation which could affect normal cells and make them susceptible to be transformed into cancer cells. Indeed, it has recently been shown that chronic inflammation is strongly associated with different human cancers such as colon, lung, and others [1,2].

Furthermore, one of the main therapeutic strategies currently used for the treatment or the prevention of cancer (chemoprevention) is to target inflammatory agents, in order to inhibit proinflammatory and neo-inflammatory factors. In this way, numerous studies have begun to use drugs to target these inflammatory pathways to prevent cell promotion

and transformation. These drug candidates have different sources such as biotechnological (monoclonal antibodies), synthetic (chemical modification), and natural bioactive compounds (including those from medicinal plants) [3]. Indeed, medicinal plants are a major and inexhaustible source of natural molecules that have already demonstrated very important anti-inflammatory and anti-cancer effects [4–7]. In this way, tomentosin could be considered as a major candidate as an anti-inflammatory and anti-cancer agent.

Tomentosin is a bioactive molecule contained in several aromatic medicinal species such as *Inula viscosa*. This molecule belongs to the chemical family of sesquiterpene lactones whose chemical structure is shown in Figure 1. Plants containing these molecules have already demonstrated tremendous biological effects such as anti-inflammatory, anti-cancer, antioxidant, and anti-microbial properties in addition to others [8,9]. Recently several correlations have been revealed between the tomentosin content and biological properties which validate the remarkable effects of this molecule, and many recent investigations have highlighted these major pharmacological actions, specifically, anti-inflammatory and anti-cancer properties [10–12].



Tomentosin

Figure 1. Chemical structure of Tomentosin.

In fact, tomentosin has an anti-inflammatory effect based on the inhibition of key pro-inflammatory and neo-inflammatory enzymes, as well as by acting on the main signaling pathways involved in the physiological and pathological inflammatory process. Moreover, this bioactive compound showed also major inhibitory effects on cancer cells directly via its different mechanisms, which involve direct and indirect targets of different checkpoints controling cell transformation such as apoptosis, cell cycle arrest, angiogenesis, and senescence [6].

Furthermore, to the best of our knowledge, there is no published review on the anticancer and anti-inflammatory effects of tomentosin. Therefore, the major objective of this work was to highlight for the first time the anti-inflammatory and anticancer properties of tomentosin and possibly to suggest it as a chemo-preventive and therapeutic agent against a certain number of human cancers.

2. Sources of Tomentosin

Tomentosin is a natural sesquiterpene lactone isolated from various aromatic medicinal plants, as reported in several studies. Indeed, Table 1 summarizes the main natural sources rich in tomentosin.

Table 1. Biological sources of Tomentosin.

Sources	Countries	Parts Used	Tomentosin Contents	References
Carpesium macrocephalum	Korea	Whole plant	nd	[13]
Cremanthodium potaninii	China	Whole plant	nd	[14]
Dittrichia graveolens	Egypt	Epigeal parts	nd	[15]
Dittrichia viscosa	Italy	Leaves	235.41 mg/g extract	[16]

Table 1. *Cont.*

Sources	Countries	Parts Used	Tomentosin Contents	References
Dittrichia viscosa	Italy	Leaves	205.80 mg/g extract	[8]
Inula viscosa	Israel	Leaves	10.6% of the total paste weight	[17]
Inula viscosa	Italy	Aerial parts	nd	[18]
Inula viscosa	Italy	Leaves	2% of dry matter	[19]
Inula viscosa	Morocco	nd	22–64% of the dry paste	[10]
Inula japonica	Korea	Flowers	nd	[20]
Inula japonica	Korea	Flowers	nd	[21]
Inula falconeri	China	Aerial parts	nd	[22]
Inula sericophylla	China	Whole plant	nd	[23]
Inula linearifolia	China	Aerial parts	nd	[24]
Inula hupehensis	China	Aerial parts	nd	[25]
Pulicaria undulata	Egypt	Aerial parts	nd	[26]
Pulicaria undulata	Saudi Arabia	Aerial parts	nd	[27]
Viguiera tucumanensis	Argentina	Aerial parts	nd	[28]
Xanthium strumarium	India	Whole plant	nd	[29]
Xanthium sibiricum	China	Aerial parts	nd	[30]
Zaluzania montagnifolia	Mexico	Leaves	0.87%	[31]
Zygophyllum album	Algeria	Aerial parts	nd	[32]

nd: not determined.

The tomentosin was identified and isolated as the main sesquiterpene, at the first time, from the *Carpesium macrocephalum* [13] and *Cremanthodium potaninii* [14]. On the other hand, among the sesquiterpene identified in the bioactive fractions of the active epigeal part of *Dittrichia graveolens* (L.) extracts from Egypt, tomentosin was identified [15]. In another study, HPLC-DAD and LC-MS (ESI) analysis of *Dittrichia viscosa* young shoots revealed the presence of tomentosin that presents a λ max at 450 nm [8]. This result was confirmed by the authors in another work [16], which detected tomentosin in *Dittrichia viscosa* at 254 nm. It was also demonstarted that Tometosin concentrations are different according to regions. Indeed, Messaoudi et al. [10] analyzed ethyl acetate and ethanolic extracts of *Inula viscosa* from three different regions of Morocco and showed the presence of tomentosin in ethyl acetate extracts at high concentrations, ranging from 22% to 64% of the dry paste. The same results were reported by two other studies [18,19], which detected tomentosin in the aerial parts of *Inula viscosa* from Italy. Lu et al. [20] and Piao et al. [21] revealed that tomentosin was a major component of *Inula japonica*, as evidenced by HPLC analysis. This sesquiterpene lactone is also identified in other *Inula* species, including *Inula sericophylla* [23], *Inula falconeri* [22], *Inula linearifolia* [24], and *Inula hupehensis* [25]. In addition, the analyses of the chemical composition of *Pulicaria undulate*, growing in Egypt and Saudi Arabia, revealed that this plant was rich in sesquiterpene lactones, including tomentosin [26,27]. It is also present in small quantities in the aerial parts of *Viguiera tucumanensis* [28] and *Xanthium strumarium* [29]. HPLC analysis of the ethanolic extract performed on the aerial parts of *Xanthium sibiricum* from China, revealed the presence of tomentosin [30]. Villa-Ruano and colleagues [31] detected this molecule (about 0.87%) in leaves of *Zaluzania montagnifolia*, while it was not detected in the flowers. Finally, tomentosin was highlighted among the sesquiterpene lactones identified in the aerial parts of *Zygophyllum album* from Algeria [32].

3. Traditional Uses of Medicinal Plants Containing Tomentosin against Cancer and Inflammation

Literature surveys based on the published studies indicated that several plant species containing tomentosin are considered as traditional remedies for the treatment of different

types of cancers and various inflammatory diseases. Indeed, *Inula viscosa* has been used as a remedy against lung cancer and inflammatory diseases by preparing the flowers of this plant as a decoction [33]. The Roots of *Xanthium strumarium* L. were prepared as an infusion and/or maceration as well as a decoction by the population of India for the same therapeutic purposes [34]. In traditional Iranian medicine, *Dittrichia graveolens* was used as an anti-inflammatory and antitumor agent [35]. In addition, the dried flowers of *Inula japonica* Thunb. have been used as Chinese folk medicine to treat inflammatory diseases [36].

4. Toxicity of Medicinal Plants Containing Tomentosin

Although 13 medicinal species contain tomentosin in their extracts (Table 1), the toxicity of these plant extracts has only been investigated for three species; *Dittrichia viscosa*, *Xanthium strumarium*, and *Xanthium sibiricum* [37–43]. According to Tchaker and collaborators [43], aqueous extracts of *Dittrichia viscosa* (the species containing tomentosin as main compound) showed no toxicity. Indeed, Ouahchia et al. [42] who tested methanolic extracts in acute and sub-chronic toxicity tests showed that this plant has no toxicity in both methods. These results corroborated the biochemical and toxicological parameters [42]. Similarly, extracts of *Xanthium strumarium* and *Xanthium sibiricum* showed no toxicity in acute, sub-chronic, and chronic tests [37–41].

5. Anticancer Activity

Chemo-resistance has become a serious problem in the management of many types of cancer. In fact, many chemotherapeutic molecules cause cell cycle arrest but not apoptosis, allowing tumor cells to repair their damaged DNA and therefore limit the efficacy of chemotherapy [44]. For the past thirteen years, all studies concerning the anticancer activity of tomentosin have been performed only in vitro on different cell lines (Table 2) [45–52].

Table 2. Anticancer effect of Tomentosin.

Cells Tested	Methods Used	Key Results	References
MCF-7 human breast cancer cells	MTT assay	Anti-proliferative activity in vitro IC_{50} values between 3.0 ± 0.1 and 31.9 ± 1.6 µg/mL	[46]
AGS gastric cancer cell line	Enzyme-linked immunosorbent assay Western blot analysis	Inhibited the cell proliferation Diminished NF-κB, TNF-α, IL-1β, and IL-6 expression levels Up-regulated the Bcl-2 and Bax expression	[50]
Human melanoma cell lines	MTT assay Flow cytometry Western blot analysis	Inhibited the proliferation of three human melanoma cells (SK-28, 624 mel, and 1363 mel) Induced the cell cycle arrest at G_2/M Induced the apoptosis Reduced protein expression of both Cdc2 (Cdk1, p34) and cyclin b1 Increased the expression level of p53 Decreased survivin expression in SK-28 cells	[45]
Human cervical cancer HeLa and SiHa cell lines	MTT assay Western blot analysis Celle cycle analysis	Inhibited the growth of SiHa (IC_{50} = 7.10 ± 0.78 µM) and HeLa (IC_{50} = 5.87 ± 0.36 µM) cell lines in a dose- and time-dependent manner Induced apoptosis and cell cycle arrest at G_2/M Increased ROS production Decreased Bcl-2 expression Cleavage of the 113kD PARP protein into 89kD fragments	[47]
Human osteosarcoma MG-63 cells	Cell migration/viability/proliferation, apoptosis, and ROS analysis assays Cell cycle analysis	Decreased cell viability and migration ability Induced apoptosis, cell cycle arrest, DNA damage, and ROS production Decreased cell viability and induced apoptosis, cell cycle arrest, and DNA damage	[48]

Table 2. *Cont.*

Cells Tested	Methods Used	Key Results	References
Human leukemia (MOLT-4) cell line	qRT-PCR Cytotoxic assay	Inhibited cell proliferation Increased intracellular ROS production Inhibited the mTOR/PI3K/Akt signaling pathway Inhibited the inflammatory transcription factors (NF-κB, TNF-α, IL-1β, and IL-6) Induced the apoptosis in MOLT-4 cells	[51]
Hepatocellular carcinoma cell lines (HepG2 and Huh-7 cells)	Western blot analysis	Activation of apoptosis signaling	[49]
Human hepatocellular carcinoma cell lines (HepG2 and Huh7 cells)	Cell counting Colony formation assay TUNEL assay Western blot analysis	Decreased the viability and suppressed the proliferation rate of HepG2 and Huh7 cells in a dose- and time-dependent manner Increased population of cells at the SubG1 and G_2/M stage Decreased population of cells at the $G_0/_1$ stage Increased apoptotic cell population and DNA fragmentation Decreased the expression levels of cell cycle-related proteins (CDK2, CDK4, CDK6, cyclin B1, cyclin D1, cyclin D2, cyclin D3, and cyclin E) and apoptosis-related proteins (PAPR-1, Bcl-2, caspase-3, caspase-7, and caspase-9) in Huh7 and HepG2 cells	[52]

Initially, Li and co-workers isolated 13 sesquiterpene lactones (5–17), from *Carpesium faberi*, in order to assess their apoptotic and anti-proliferative activities against human breast cancer (MCF-7) cells using the MTT test [46]. They noted that all the molecules, with the exception of tomentosin 17, showed anti-proliferative activities, with IC_{50} values varying between 3.0 ± 0.1 and 31.9 ± 1.6 µg/mL.

The mechanisms of anticancer drugs move from the subcellular to the molecular level by activating or inhibiting gene expression across the cellular levels (activation, inhibition, and interference with signaling pathways). Indeed, the mechanistic studies carried out on tomentosin have highlighted certain actions at the cellular level and especially actions at the molecular level. In the following sections we will discuss the anticancer action of tomentosin at the cellular and molecular level.

5.1. Cellular Level

A Chinese research team investigated the cellular action mechanisms of tomentosin on human gastric cancer cells (GCCs) AGS [50]. They assessed the impact of tomentosin (20 µM) on inflammatory mediators, intracellular ROS, mitochondrial membrane potential (MMP), and cell proliferation/migration/adhesion/apoptosis/oxidative stress. Besides, the Western blot analysis method and enzyme-linked immunosorbent assay have been used to analyse apoptotic and inflammatory molecular markers. Accordingly, apoptosis was initiated by an increase in ROS synthesis in AGS cells following the action of tomentosin. Indeed, after 24 hours of treatment, an up-regulation of the levels of antioxidant enzymes (CAT, SOD, and GSH) was observed, which illustrates that this molecule improved the expression of these enzymes in cancer cells by scavenging intracellular ROS. Furthermore, evaluation of MMP showed that cancer cells tested in this study absorbed high levels of tomentosin compared to control cells.

5.2. Molecular Level

At this level, the first work was carried out in 2008 to discover the molecular mechanism of tomentosin action against cell lines of human melanoma, a very aggressive tumor resistant to drug treatments, by evaluating its capacity to induce G_2/M arrest and apoptosis [45]. Therefore, tomentosin isolated from *I. viscosa* leaves inhibited the proliferation of three cell lines, namely 1363 mel, 624 mel, and SK-28 in a dose-dependent manner. In order

to better understand this mechanism of action, SK-28 was chosen to be the representative cell model.

This sesquiterpene lactone caused apoptotic cell death via induction of G_2/M cell cycle arrest, which was also confirmed by detection of caspase-3 activation, and modifications of the mitochondrial membrane permeability and the membrane phospholipids. Moreover, treatment with tomentosin reduced the expression level of the cyclin B1 protein involved in mitosis and of Cdk1 (also called Cdc2), a protein essential for the proper development of the cell cycle (Figure 2). On the other hand, a transcription factor (p53 protein) promoting the expression of genes involved in the inhibition of CDKs and the repair of cellular damage has also been verified. $p21^{waf1}$ is one of those genes that inhibit the cyclin B1/Cdc2 complex activity resulting in cell cycle arrest in the G_2 phase [44]. In this study [45], and following two hours of treatment with tomentosin, $p21^{waf1}$ expression levels were measured in SK-28 cells to verify p53 activity. Effectively, a high expression of this gene as well as an increase in Cdc2 phosphorylation (allowing binding to cyclins) were recorded, which consequently reduced the expression of the cyclin B1/Cdc2 complex 24 hours after treatment. Interestingly, a marked increase in the phosphorylation of the p53 protein at Ser15 (phospho-p53) was observed in a concentration-dependent manner, which, according to Shieh et al. [53], was able to stabilize the p53 protein and improve its trans-activating power.

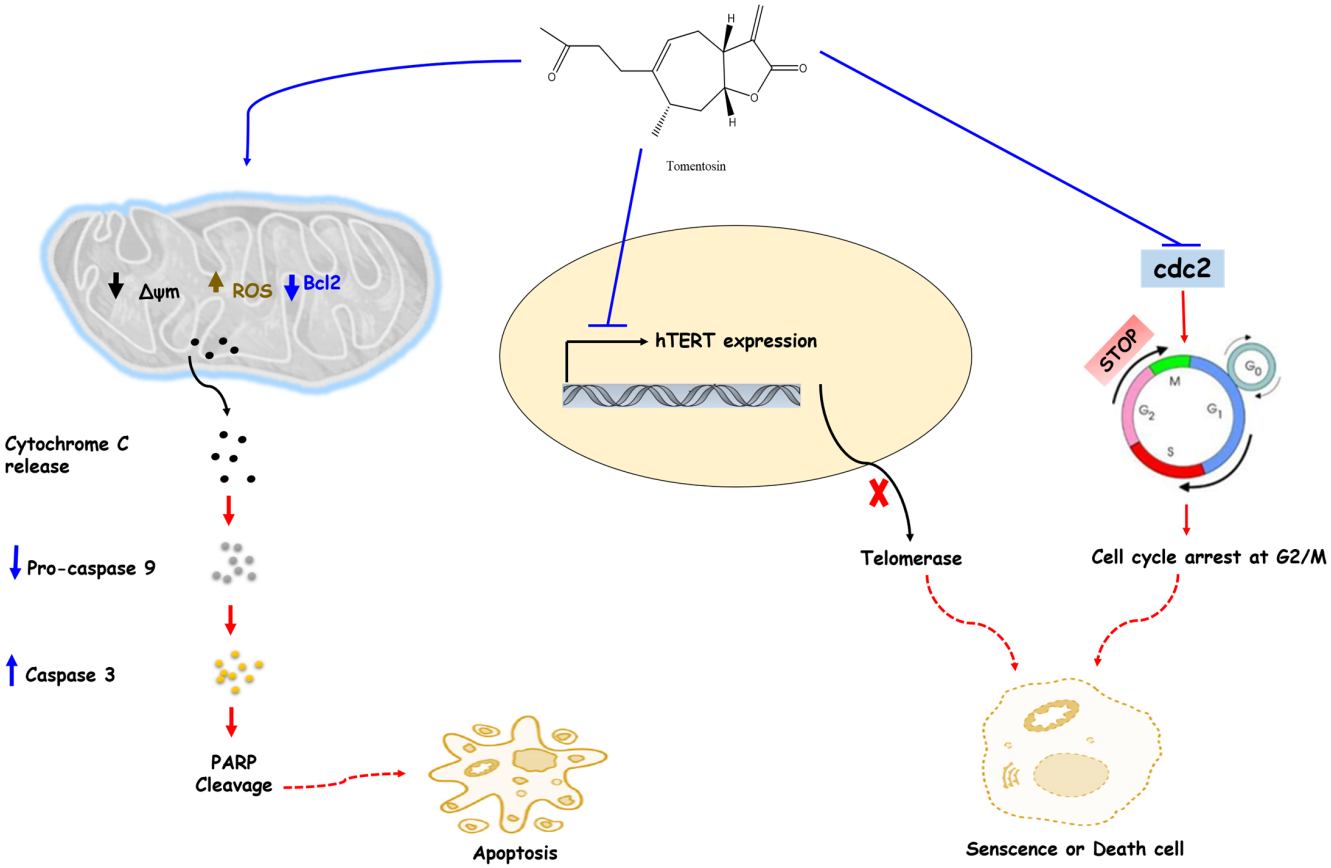


Figure 2. Anticancer mechanisms of Tomentosin.

Additionally, in order to unveil the mechanisms responsible for this apoptotic death, the authors examined the effect of this molecule against the survival protein, survivin, contributing to the resistance of cancer cells to chemotherapeutic agents inducing apoptosis [54,55]. The expression of this apoptosis inhibitor protein increases during the transformation of malignant cells [56]. Its anti-apoptotic potency is associated with its ability to inhibit the effector caspases-3 and -7 [57]. Indeed, inhibition of survivin can improve the effectiveness of chemotherapy and promote natural apoptosis in cancer cells [58]. The inhibition

of this anti-apoptotic protein was expressed in this work by a sharp decrease in its levels after 24 h of treatment.

The promising results of this study suggest that tomentosin may sensitize cancer cells to apoptosis, particularly in tumors resistant to apoptosis.

The anticancer potency of a compound can also be studied through its ability to shorten chromosomal telomeres [59]. Hence the objective of Merghoub and collaborators who, in addition to this ability examined for the first time with tomentosin, they studied its anti-proliferative effect on human cervical cancer cell lines (HeLa and SiHa cells) [47]. Indeed, the anticancer activity in this study was highlighted by several ways, namely cell growth inhibition, telomeric overhang elongation, morphological change expression, mitochondrial membrane potential ($\Delta\Psi m$) depolarization, ROS generation, apoptosis-related proteins expression, caspase-3 activity, and cell cycle analysis) (Figure 2).

Initially, tomentosin dose-dependently inhibited the proliferation of SiHa (IC_{50} = 7.10 ± 0.78 μM) and HeLa (IC_{50} = 5.87 ± 0.36 μM) cells after 4 days of treatment. In order to verify this inhibition, DNA staining using fluorescent markers (Hoechst 333242) was carried out to reveal, consequently, after 24 h of treatment an increase in the number of apoptotic cells (apoptotic bodies and nuclear chromatin condensation) in a dose and time dependent manner. Depending on $\Delta\Psi m$ depolarization and measurement of ROS production, this apoptosis may be induced by the apoptotic pathway mediated by mitochondria in the cancer cells studied (Figure 2). Afterwards, it was confirmed that the telomeres constitute a specific target for this molecule at a concentration of 20 μM; by decreasing the telomeric overhang length for SiHa (63.32 ± 6.56%) and HeLa (74.11 ± 8.16%) cells and by inducing high sensitivity in foetal fibroblasts JW10 cells. Furthermore, this cytotoxic effect was accompanied by an increase in the activity of caspase-3 (cleavage of PARP and pro-caspase-3) the expression of the tumor suppressor p53 as well as a decrease in the expression of the anti-apoptotic protein Bcl-2 in both cell lines. Moreover, the proportion of SiHa and HeLa cells in G_2/M phase increased considerably with increasing concentrations during 48 hours of treatment, as previously reported, indicating disturbances in cell cycle progression.

In order to study the anticancer activity of tomentosin on osteosarcoma, Korean researchers have evaluated the anti-carcinogenic effects of this molecule on MG-63 cells by analyzing apoptosis, ROS generation, and cell proliferation/viability/migration [48]. Therefore, a decrease in migration capacity was noted in MG-63 cells with regeneration of intracellular ROS, which induced DNA damage, cell cycle arrest, and apoptosis in addition to decreased cell viability. This suggests that the cytotoxic effects of tomentosin in MG-63 cells are primarily attributed to the induction of intracellular ROS.

In addition to the cellular impact recorded above by Yang et al. [50], this natural sesquiterpene lactone has also shown several positive results via the decrease in the activity of NF-κB and the expression levels of pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β), which therefore increased the expression of Bcl-2, a pro- or anti-apoptotic protein. The pro-inflammatory transcription factor, NF-κB, effectively regulates genes involved in angiogenesis, tumor invasion, drug resistance, and metastasis [60,61]. Chemotherapy can stimulate activation of this pathway, inducing resistance to apoptosis and expression of anti-apoptotic genes [62]. The induction of the apoptotic pathway in this work was carried out by inhibiting inflammatory mediators through the modulation of gastric cancer cell proteins.

Very recently, Yang et al. [51] explored the same anticancer properties of this bioactive compound against the human leukemia cell line MOLT-4. After 24 hours of treatment, the authors recorded an important cytotoxic power (IC_{50} = 10 μM) associated with intracellular ROS production and induction of intrinsic pathways in MOLT-4 cells. They also noted, in treated MOLT-4 cells, the onset of apoptosis confirmed by fluorescent microscopic studies, as well as up-regulation of caspase-3 and Bax mRNA expression, and down-regulation of Bcl-2 and of cyclin D1 expression revealed by qRT-PCR assay. Likewise, treatment with this molecule significantly inhibited inflammatory transcription factors (IL-6, IL-1β, TNF-α,

and NF-κB) and the protein expression of m-TOR/PI3K/Akt signaling pathway in tumor cells. This pathway is one of the complex signaling pathways in cancer cells responsible for their growth, proliferation, and survival [63]. Interestingly, the pro-apoptotic pathway in this experiment was stimulated by caspase induction while the anti-apoptotic pathway was stimulated by Bcl-2 inhibition in MOLT-4 cells.

On the other hand, regarding hepatocellular carcinoma (HC), it has been explored by two studies [49–52] by treating two human HC cells (HepG2 and Huh7) with different doses of tomentosin. Although the first study [49] did not investigate the effect of this compound in detail, it showed activation of apoptosis signaling. While concerning the second study [52], several promising results, aiming to elucidate the underlying mechanism(s) of this cytotoxic activity in the HepG2 and Huh7 cell lines have been reported. Indeed, cell cycle control showed an increased cell population in the Sub-G_1 and G_2/M phase (Figure 2), and a reduced cell population in the $G_{0/1}$ phase in the treated cancer cells. Additionally, a suppression of the proliferation levels of Huh7 and HepG2 cells and a decrease in their viability were noted in a dose- and time-dependent manner. Additionally, Huh7 and HepG2 cells treated with tomentosin expressed DNA fragmentation and a high apoptotic cell population. During 48 hours of treatment, tomentosin (0, 20, and 40 μM) decreased the expression levels of cell cycle-related proteins (CDK2, CDK4, CDK6, cyclin B1, cyclin D1, cyclin D2, cyclin D3, and cyclin E) and apoptosis-related proteins (PAPR-1, Bcl-2, caspase-3, caspase-7, and caspase-9) in Huh7 and HepG2 cells in a dose-dependent manner (Figure 2). Taken together, these observations suggest that the observed cell cycle arrest was mediated by the regulation of cyclins and CDK inhibitors and that cell apoptosis was promoted by activation of the intrinsic apoptosis pathway induced by tumor suppressive transcriptional factors and caspase. In this work, the authors also studied the expression levels of MAPKs, which can modulate many physiological processes in cancer cells. The ERK and AKT signaling are activated in most human tumors, making them therapeutic targets. Indeed, ERK is involved in cell differentiation [64] and extrinsic/intrinsic apoptotic pathway activation [65]. The expression level of phosphorylated (p) AKT was decreased in Huh7 and HepG2 cells treated with tomentosin, while the expression level of pERK was increased in a dose-dependent manner. From these findings, it can be inferred that the impact of tomentosin on cell survival and cell cycle of hepatocellular carcinoma is attributed to the ERK signaling pathway.

The results of all these studies highlight the potential of tomentosin as a pharmacological candidate capable of exerting multi-target chemotherapeutic actions against the tumor microenvironment. However, we noted the absence of pharmacodynamic studies evaluating molecular action (ligan/receptor relationship between tomentosin and subcellular targets). Such investigations provide mechanistic responses on membrane receptors responsible for cellular and molecular changes. Further studies (in vivo and toxicity tests) are also needed in order to allow the use of this molecule in clinical trials.

6. Anti-Inflammatory Effect

Inflammation is clinically defined as a pathophysiological process characterized by redness, edema, fever, pain, and loss of function. Although the steroidal and non-steroidal anti-inflammatory drugs currently in use treat acute inflammatory disorders, these conventional drugs have failed to treat some chronic inflammatory disorders [66]. Thus, natural products have acquired great importance as potential anti-inflammatory agents. In this sense, certain studies (in vivo and in vitro) have evaluated this property with tomentosin (Table 3) according to different protocols [67–71].

Table 3. Anti-inflammatory effect of Tomentosin.

Cells Tested/Animal Models	Methods Used	Key Results	References
Peripheral blood mononuclear cells (PBMCs)	In vitro effect on the pro-inflammatory cytokines secretion from human PBMCs Western blot analysis	Decreased production of IL-2, IL-1β, and IFNγ Slightly increased secretion of TNF-α Reduced the protein level of p65/RelA of NF-κB and STAT1 by proteosomal degradation	[67]
RAW264. 7 cells	Effect on the production of inflammatory mediators as well as on the activation of NF-κB and MAP kinase	Decreased the NO production by suppressing the protein expression of iNOS Decreased the PGE_2 production by suppressing the protein expression of COX-2 Reduced the release of pro-inflammatory cytokines Suppressed the phosphorylation of MAP kinases	[68]
Healthy male Sprague Dawley rats (in vivo) SH-SY5Y, human neuroblastoma cells (in vitro)	Inhibited neuro-inflammation in in vivo and in vitro models qPCR analysis MTT assay	Decreased neurological deficient Reduced inflammatory cytokine levels Inhibited the NLRP3 inflammasome activation in rats Inhibited the pro-inflammatory cytokines in OGD-R induced SH-SY5Y cells	[69]
Male C57/BL6 mice	MPTP-stimulated neuroinflammation in mice ELISA test Western blot analysis	Decreased pro-inflammatory cytokine levels Inhibited the TLR_4/NF-κB signaling pathway Prevented inflammation-mediated neuronal cell damage Reduced glial cell damage Normalized ganglion layers	[71]
BALB/c mice (mouse model of sepsis)	Effect against LPS-induced acute lung injury via the suppression of TLR_4/NF-κB pathway Western blot analysis	Suppressed the status of pro-inflammatory markers Reduced the activation of iNOS, MPO, COX-2, and PGE_2 in the lung Down-regulated the TLR_4/NF-κB signaling pathway	[70]

The first work that began to investigate this dates back to 2010, when tomentosin was extracted and purified from the leaves of *Inula viscosa* (Compositae), a plant known for its anti-inflammatory power [67]. Indeed, the anti-inflammatory effect was tested in vitro on the production of pro-inflammatory cytokines (IFNγ, TNF-α, IL-2, IL-6, and IL-1β), small proteins secreted by cells in response to various stimuli. They are synthesized in response to an attack, allowing communication between cells in order to develop a response depending on the nature of the signal detected and thus promoting tissue repair and regeneration. However, when the production of these proteins is excessive and/or prolonged, tissue damage can occur. The results of this study, conducted by Abraham and colleagues, showed that tomentosin reduced the secretion of IFNγ (EC_{50} = 2.2 ± 0.5 μM), IL-1β (EC_{50} = 0.5 ± 0.2 μM), and IL-2 (EC_{50} = 0.7 ± 0.8 μM).

Anti-inflammatory agents can suppress inflammation *via* actions at the level of systemic mediators but also via actions at subcellular and molecular level passing through actions at cellular level (actions on different inflammatory pathways). Studies carried out so far have revealed anti-inflammatory actions expressed at the cellular and molecular level. In the next section, we will discuss in order the cellular and molecular actions of tomentosin.

6.1. Cellular Level

In a study realised by Park et al. [68], tomentosin was isolated from Inulae flos to assess its effects (in vitro) on the production of inflammatory mediators (NO, iNOS, PGE_2, COX-2, TNF-α, and IL-6) as well as on the activation of NF-κB and MAP kinase in RAW264.7 macrophage cell line. Moreover, MAPKs are directly involved in cell proliferation and inflammation, in response to certain external signals such as cytokines. It is the main signaling pathway in the cell. Therefore, tomentosin inhibited the production of inflammatory mediators involved in mediating the inflammatory response; by suppressing the activation of MAP kinase and NF-κB pathways in RAW264.7 cells.

In fact, under normal physiological conditions, NO and NOS can play a major role in the inflammatory process, by acting as anti-inflammatory agents. However, an overproduction of these molecules in abnormal situations can induce inflammatory diseases such as inflammatory joint disease [72]. During an inflammatory reaction, a rapid synthesis of COX-2 occurs followed by the production of PGE_2, which can be protective or deleterious, in particular on the intestinal epithelial barrier. In this context, an old study recorded a correlation between the severity of ulcerative colitis in patients and high concentrations of PGE_2 [73].

In a recent study, a Chinese research team examined the inhibitory power of this molecule on neuroinflammation in in vivo (Sprague-Dawley rats) and in vitro (SH-SY5Y cells) models [69]. In fact, cerebral ischemia-reperfusion was induced in animals, which then received tomentosin (25 and 50 mg/kg b.w) for seven days to analyse brain edema, as well as the levels of inflammatory cytokines, antioxidants, and NLRP3 signaling proteins. For the in vitro study, human neuroblastoma cells were pre-treated with tomentosin in an OGD-R model. Accordingly, the in vivo neuroprotective power of tomentosin was determined through enhancement of antioxidant potential, inhibition of inflammatory cytokines, and NLRP3 signaling, with histopathological confirmation. These results are in line with those obtained in vitro, which reinforces the use of this molecule in the therapy of ischemic stroke. This action against neuroinflammation has been explored very recently against another form of neurodegenerative disease, Parkinson's disease (PD) [71], which was stimulated in C57/BL6 mice having received MPTP (20 mg/kg) and treated simultaneously with tomentosin isolated from *I. viscosa* following a 15-day experimental protocol. In order to confirm this neuroprotective property, several tests and methods were carried out in vivo, namely psychomotor tests, measurement of ROS, SOD, and pro-inflammatory cytokines levels, analysis of TLR_4/NF-κB pathway expression, and finally histopathological analysis of brain tissue. The authors found that the compound of their work exerted several positive effects against inflammatory disorders related to PD, mainly motor disturbances, cellular damage (neurons and glial cells), as well as high levels of ROS and pro-inflammatory mediators.

6.2. Molecular Level

According to the work of Abrham et al. [67], already cited in the previous section, a decrease in the expression of NF-κB p65 subunit involved in the immune response has been reported due to proteasomal degradation. Indeed, the activation of NF-κB can induce the transcription of pro-inflammatory cytokines or that of iNOS. Recently, NF-κB has become a major therapeutic target for the development of new anti-inflammatory drugs.

Recently, researchers have started to investigate the activity of this sesquiterpene lactone in vivo. Indeed, Zhu et al. [70] induced a severe inflammatory disorder (acute lung injury) via suppression of the TLR_4/NF-κB signaling pathway by lipopolysaccharides (LPS) in BALB/c mice. Subsequently, these sepsis animals were treated with tomentosin (20 and 25 mg/kg) using dexamethasone as the reference drug. Histopathological analysis was performed to examine lung tissues. The studied compound has shown promising results in decreasing the severity of pulmonary edema and the levels of immune cells (neutrophils, lymphocytes, and macrophages) in the bronchi and pulmonary alveoli. It also down-regulated the TLR_4/NF-κB pathway and inhibited the biosynthesis of pro-inflammatory markers in the lungs. Consequently, tomentosin could be considered in the management of sepsis.

To justify the choice of the TLR_4/NF-κB pathway as a therapeutic target in the studies already cited, it is necessary to underline its role in the case of inflammatory disorders. In fact, a cascade of proteins involved in the MAP kinase and NF-κB pathways is activated during inflammation, then allowing macrophages, activated by the stimulation of their TLR_4 receptors, to participate in the resolution of the inflammatory response.

Despite the findings of all the investigations carried out since 2010 until the appearance of this review, proving the anti-inflammatory activity of tomentosin, toxicity tests have been

shown to be necessary in order to verify its safety and therefore validate its therapeutic use in clinical trials.

On the other hand, the association between inflammation and other pathologies, in particular cancer, has attracted the attention of several researchers. Chronic inflammation is associated with malignant tumors, which is why it is important to prevent inflammation-induced neoplastic formation.

7. Conclusions and Perspectives

The present work has highlighted anti-inflammatory and anti-cancer effect. This molecule exerts its action at subcellular, and molecular levels via inhibiting and/or activating enzymes involved in inflammation and signaling pathways in cancer cells which can activate NF-κB (main transcirptional factor in inflammation and cancer processess). Indeed, it was highlighed in this review that tomentosin directly exhibits major effects on cancer cells via the inhibition of a number of signaling pathways that control checkpoint molecules, namely the inhibition of caspase, telomerase, and the respiratory chain of cancer cells. However, the research work that has been carried out often concerns in vitro and sometimes in vivo studies. For this reason, the validation of this molecule for chemoprevention and cancer treatment requires major investigations concerning, first of all, the pharmacodynamic validation (specific action of this molecule) but also the validation of pharmacokinetics and administration pathways of this molecule via in vivo investigations in laboratory animals. Moreover, the toxicity of this plant (acute and chronic) is essential to validate its importance and suggests its candidacy for chemoprevention and treatment.

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Abbreviations

AKT	Protein kinase B
Bcl-2	B-cell lymphoma 2
CAT	Catalase
CDK	Cyclin-Dependent Kinase
COX-2	Cyclooxygenase-2
DNA	Deoxyribonucleic acid
EC_{50}	Half-maximal effective concentration
ELISA	Enzyme-Linked Immunosorbent Assay
ERK	Extracellular signal-Regulated Kinase
GCC	Gastric Cancer Cell
GSH	Glutathione
HC	Hepatocellular Carcinoma
HPLC-DAD	High-performance Liquid Chromatographic method with Diode-Array Detection
IC_{50}	Half-maximal inhibitory concentration
IFNγ	Interferon gamma
IL	Interleukin
iNOS	Inducible Nitric Oxide Synthase
LC-MS (ESI)	Liquid chromatography-mass spectrometer (electrospray ionisation)

LPS	Lipopolysaccharides
MAPK	Mitogen-Activated Protein Kinase
MMP	Mitochondrial Membrane Potential
MPO	Myeloperoxidase
MPTP	1-methyl-4-Phenyl-1,2,3,6-Tetrahydro-Pyridine
mTOR	Mammalian Target of Rapamycin
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NLRP3	Nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3
NO	Nitric Oxide
OGD-R	Oxygen-glucose deprivation/reperfusion
PARP	Poly(ADP-ribose) polymérase
PBMC	Peripheral Blood Mononuclear Cells
PD	Parkinson's Disease
PGE_2	Prostaglandin E_2
PI3K	Phosphatidylinositol-3-kinase
qRT-PCR	Quantitative Real-Time Polymerase Chain Reaction
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
STAT1	Signal Transducer and Activator of Transcription 1
TLR4	Toll-like Receptor 4
TNF-α	Tumour Necrosis Factor-α
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling

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