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SHORT REVIEW

Insight Into the Role of Alkaloids in the Different Signalling Pathways of Cholangiocarcinoma

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Abstract

Throughout the biliary tree, a variety of cells give rise to cholangiocarcinomas, a broad group of malignancies. The fact that these tumours are silent and asymptomatic, especially in their early stages, seriously impairs the effectiveness of available therapeutic options and contributes to their poor prognosis. Over the past few years, increased efforts have been made to identify the aetiology and signalling pathways of these tumours and to create more potent therapies. Since alkaloids are more potent and effective against cholangiocarcinoma cell lines, they have gained importance in the treatment of cholangiocarcinoma. In cell lines with cholangiocarcinoma, they promote apoptosis. and restrict the spread of cells, departure, and development. This review highlights the recent developments in the study of CCA, primarily concentrating on the regulation of the signalling pathway and revealing alkaloids demonstrating strong anti-cholangiocarcinoma efficacy, providing researchers with a rapid approach for the future development of powerful and efficient pharmaceutical compounds.

Keywords: Alkaloids, Cholangiocarcinoma, Risk Factors, Signalling Pathway

1. Introduction

Cholangiocarcinoma (CCA) is a form of malignancy that develops when hepatocytes, the epithelium covering the bile duct's intrahepatic and extrahepatic parts, undergo neoplasticism[1,2]. Historically, cholangiocarcinoma has always been viewed as a "rare" tumour[3], particularly in Western nations; instead, throughout the previous fifteen years, its incidence has steadily expanded around the globe, today, it's considered the second-highest prevalent kind of initial cancer in malignancy (15–20 % of instances), behind hepatocellular carcinoma (HCC)[4]. Cholangiocarcinoma is an extremely serious disease with a very dismal outcome that was originally identified by Steiner and Higginson. In terms of its anatomy, pathological conditions, and medical characteristics, cholangiocarcinoma is a highly unique cancer. The greatest rate of CCA is seen in Northeast Thailand, where there are 100 cases per 100,000 men and 50 cases per 100,000 women. In comparison, there are just 1-2 instances per 100,000 people in the Western world, due to various risk factors associated with different racial, genetic, and environmental backgrounds[5]. The second-order bile ducts play anatomical partitions between the two categories of CCA, which are traditionally referred to as intrahepatic (iCCA) and extrahepatic (eCCA) cholangiocarcinoma[6]. According to the position of the cystic duct, extrahepatic cholangiocarcinoma

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is classified into hilar (Klatskin) or perihilar, distal tumours. In particular, perihilar cholangiocarcinoma develops in the right and/or left hepatic ducts or at the point of intersection; distal cholangiocarcinoma affects the common bile duct; and iCCA develops the second-level liver ducts upstream[7]. The majority of cholangiocarcinomas (60 %) are hilar, accompanied by distal (20–30 %) and iCCA (5–10 %) tumours, while there has been a steadily rising prevalence and mortality for intrahepatic carcinoma worldwide[8].

Fibroblast growth factor receptor 2 (FGFR) fusions, which are found in 10–15 % of intrahepatic carcinomas but nearly never in extrahepatic carcinomas, are one of the most promising categories of targets[9]. The Erythroblastic leukaemia viral oncogene homologue (ErBb) family consists of the receptor tyrosine kinase-epidermal growth factor receptor (RTK-EGFR), also called erythroblastosis oncogene B1, erythroblastic oncogene B2, erythroblastic oncogene B3, and erythroblastosis oncogene B4. The ErBb family is linked with the progression of cholangiocarcinoma through the control of cellular networks, which are strengthened during tumour development, metastasis, and chemoresistance[10]. Furthermore, the ErBb family's overexpression and somatic mutation-mediated changes imparted on cholangiocarcinoma and other malignancies increase tumour aggressiveness and chemoresistance by influencing the tumour microenvironment[11]. Natural products are regarded as significant sources of novel chemicals, including leads and new medications[12]. The creation of diverse anticancer medicines from alkaloids is the main focus of plant-based drug exploration. Alkaloids are most prevalent in biological material and are mostly found in particular groups of flowering plants. Alkaloids come in over 3000 different varieties[13]. Numerous alkaloids have been identified as crucial in the prevention of CCA through regulation, prevention of the growth of cells, and modification of the level of cellular autophagy.

This review emphasises recent developments in the study of CCA, primarily concentrating on the mechanisms of the signalling pathway and revealing the effective alkaloids displaying potent anti-cholangiocarcinoma activities, which will help scientists quickly develop the concept for future potent and efficient pharmaceutical compounds.

2. Epidemiology and Risk Factor

CCA is one of the three primary malignancy origins and ranks as the sixth most common kind of malignancy worldwide[14,15]. Cholangiocarcinoma is the second-highest prevalent recurrent carcinoma of the hepatocyte behind HCC[16,17]. Biliary tract cancer accounted for approximately 13% of the 7.6 million annual deaths caused by tumours in the world, 3% of the 560,000 annual cancer-associated fatalities in the United States (U.S.) whereas cholangiocarcinoma is responsible for 10–20 % of the fatalities from biliary liver cancer. CCA occurrence rates differ dramatically throughout the world due to variances in local risk factors as well as genes. China and Japan have the largest fatality rates worldwide, followed by India and East Asia, with Australia reporting the lowest rates. The prevalence of cholangiocarcinoma in the U.S. was observed to be 0.95/100,000 for intrahepatic carcinoma and 0.82/100,000 for extrahepatic CCA[18]. In Europe, the incidence of intrahepatic cholangiocarcinoma was greater as compared to Central and Northern Europe[19]. Recently, more studies were carried out on the fatality and prevalence rate of CCA, which revealed in Italy, where between 1980 and 2003 there was a 40-fold increase in intrahepatic cholangiocarcinoma mortality, the extrahepatic cholangiocarcinoma fatality trend was balanced and may have been somewhat reduced over the past ten years[20]. Thus, according to reports in other countries, the fatality trend is higher in Italy for CCA mostly, due to the IH-CCA manifestation, which indicates the distinct aetiology and adverse condition for intrahepatic cholangiocarcinoma and extrahepatic cholangiocarcinoma[19]. CCA is a rare biliary system tumour that accounts for up to 20% of all hepatic cancers[21]. Most of the CCA patients are over the age of 65 in the West, where the median age of assessment is 50 years[22]. Numerous patients receive diagnoses of incurable diseases with extremely dismal prognoses, with an average estimated mortality of 3-6 months. The 5-year overall mortality rates for CCA stages 3 and 4 are 10 and 0%, respectively[23]. The global CCA incidence rates, per 100 000 (100), in decreasing order, have been shown in Table 1.

Table 1. Overall CCA occurring assess, per 100 000 (100), in decreasing order[24]

Region	Age-standardized incidence rate/100 000 population
Hong Kong	2.3
Taiwan	4.7
Italy	3.4
Germany	3
United Kingdom	2.2
France	1.3
Australia	0.4
United States	1.6
Singapore	1.5
Denmark	1.3
Spain	0.5
Switzerland	0.5

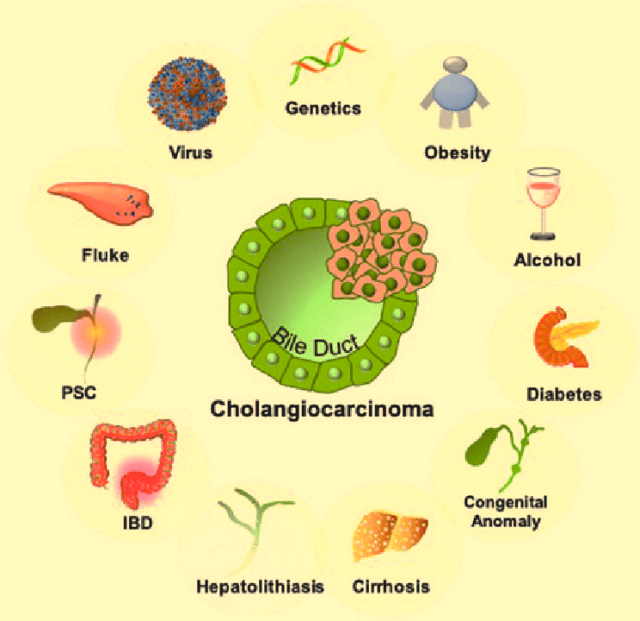

Figure 1. Risk factor of CCA.

Various diseases, including hepatic/choledocholithiasis, hepatitis B, cirrhosis of the liver, obesity, Diabetes Mellitus (DM), congenital hepatic fibrosis, and Primary Sclerosing Cholangitis (PSC)[25], have been related to the progression of CCA. Only a small percentage of cholangiocarcinoma trends are connected to an established risk factor, while the majority of instances are unpredictable[26]. Parasitic infections, including Clonorchis sinesis[27,28] and Opisthorchis viverrine[29], PSC[30], biliary tube tumours, hepatolithiasis, viral hepatitis, and poisons, are all risk factors for CCA, as shown in Figure 1[31]. Cirrhosis[32], Inflammatory Bowel Disease (IBD)[33], adiposity, diabetes, gallstones, alcoholism, and smoking are all potential risk factors for CCA. Several risk factors are connected to IH-CCA and others to EH-CCA[34].

3. Signaling Pathway

Chronic inflammation is one of the causes of cancer involving CCA. Moreover, a fraction of CCAs develop after chronic cholangitis, biliary damage, or obstruction. Normal cholangiocytes may undergo mitogenic responses in the presence of pro-inflammatory cytokines, proliferation components, and hazardous cholic acid, thus encouraging the deposition of mutant cells as well as uncontrollable cell growth. In this way, several signalling pathways are unregulated, which helps in uncontrollable cell growth, survivability, maturation, aggression, and progression, thus nourishing CCA development[35]. These are some of the pathways described below.

3.1 Inflammation-Associated Pathway

Many of the proinflammatory cytokines, including Tumour Necrosis Factor-α (TNF-α)[36], interleukin-6[37], Tumour Necrosis Factor-β (TNF-β)[38] and Platelet-Derived Growth Factor (PDGF)[39], are released by mesenchymal stem cells and cholangiocarcinoma cells, having a significant role in paracrine and autocrine ways of encouraging and maintaining carcinogenesis[40,41]. CCA has induced nitric oxide synthase, which is absent in normal bile ducts. The stimulation of the nitric oxide synthase results in the immoderate deposition of Nitric Oxide (NO) and nitrosative stress, which elevate deoxyribonucleic acid (DNA) destruction sequences and especially obstruct DNA reformation activity, thus enabling tumour development and growth[42]. Inducible Nitric Oxide Synthase (iNOS) stimulation and cholic acid formation may also promote the stimulation of inflammatory cytokines including cyclooxygenase-2, further resulting in the formation of cell growth

and cell death by a mechanism dependent on the prostaglandin E2-dependent initiation of the protein kinase B, Epidermal Growth Factor (EGF) pathways[43].

Interlekin-6 (IL-6) helps in cholangiocarcinogenesis[44] by increasing its level in cholangiocarcinoma cells in comparison to normal cholangiocytes and cancer cells and bile from patients with cholangiocarcinoma[45]. IL-6 promotes the stimulation of Janus kinase 1/2 (JAK1/2) by its interaction with the glycoprotein 130 (gp130) receptor, thus resulting in the phosphorylation and stimulation of STAT3 as shown in Figure 2, which ultimately initiates the transcription of proteins that help in cell proliferation and development. CCA development may be stabilised by the IL-6-dependent stimulation of extracellular signal-regulated kinase 1/2, which causes the attraction of ribosomal S6 kinase 1 and phosphorylation of the transcription factor C, ultimately enhancing progranulin regulation. Enhancing interleukin-6 concentration in cholangiocarcinoma, resulting in the prevention of cell cycle damage, particularly via mitogen-activated protein kinase pathway, stimulating mitogenic p38 mitogen-activated protein kinases (p38 MAPK) pathway, thus enhancing the level of cell cycle regulator p21[46].

The cytokines tumour necrosis factor-β (TGF-β) plays a significant role in numerous dependent context cell fate decisions. TGF-β can also be produced or reacted by almost all cell types, and there are several TGBβ cellular receptors, co-receptors, and various TGF-β family members. In cases of liver fibrosis, TGF-β can promote the stimulation of Hepatic Stellate Cells (HSC). TGF-β can also activate the epithelial cell and may potentially have cytostatic or cancer-promoting effects, which further influence the pathology of cholangiocarcinoma in a complicated way[47].

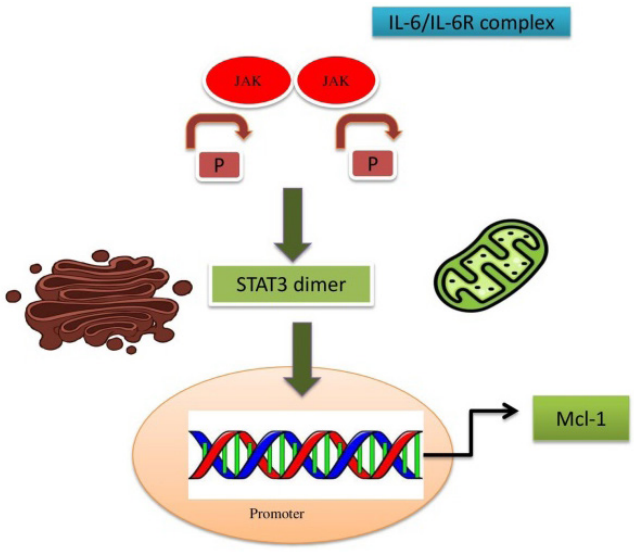

Figure 2. Schematic illustration of IL-6/STAT3 signalling.

3.2 Evolutionary Pathways

Many evolutionary pathways, such as Notch, Wingless-related integration site (Wnt), and Hedgehog (Hh), that are typically active during oogenesis are regenerated during liver damage and healing.

The Notch pathway is started when ligands engage the Notch receptor, triggering a string of proteolytic events that cause the cell membrane to secrete Notch intracellular domain 1. After that, the Notch intracellular domain 1 (NICD1) is moved into the embryo, where it plays a key role as a transcriptional activator for regulating cellular proliferation, biliary healing, and development. Notch1, 3, and 4 receptors are present for regulation in most human intrahepatic cholangiocarcinoma tumours. Notch3 activation was associated with the seriousness of the illness and enhanced cell viability via activating the phosphoinositide-3-kinase/protein kinase B (PI3K/AKT) pathway[48].

The Wnt/β-catenin route is a highly preserved evolutionary route which helps in the regulation of hepatobiliary expansion, cell progression, and survival during oogenesis and liver improvement during the duration of maturity[49]. The discharge of wingless-related integration site (Wnt) ligands such as Wnt family member 7B (Wnt7B) and wnt family member 10A (Wnt10A) occurs by the activation of monocytes in the tumour microenvironment (TME), and this ligand further attaches to the curled site, low-density lipoprotein receptor-related protein (LRP5/6) coreceptors in cholangiocarcinoma cells, thus enhancing the Wnt signalling that is found in the CCA and resulting in the nuclear translocalization of catenin and advancement of cell growth and development[50].

The Hedgehog signalling pathway (Hh) pathway[51] regulates cell viability, division, embryogenesis, proliferation, and self-renewal[52]. Hh ligand interacts with the patched 1 receptor, smoothing out the intracellular signal, which culminates in the initiation of glioma-associated transcription of genes, which further undergoes nuclear translocation and stimulates the gene's transcription, promoting division, migration, and mortality. In most of the CCA cases, the Hh

pathway was stimulated, further, it was associated with reducing oxidative stress, reducing disease-free survival, and enhancing growth in two autonomous adherent individuals[53].

3.3 Pathway of Receptor Tyrosine Kinase

During cholangiocarcinogenesis, tyrosine kinase is a growth factor that is found to be activated. These are some of the growth factors which is described below as shown in Figure 3.

3.3.1 Fibroblast Growth Factor Receptor (FGFR)

FGFRs, containing FGFR1-4, a group of Receptor Tyrosine Kinase (RTKs) which are essential for the regeneration of tissue during embryonic development and cancer angiogenesis and propagation. Depending on the biological system, the extracellular matrix (ECM) of FGFR may interact with 22 distinct fibroblast growth factors[54]. Fibroblast Growth Factors (FGF) interact with the FGFR and generate conformational alteration, which results in fibroblast growth factor receptor dimerization and mutual cross-phosphorylation of cytoplasmic tyrosine residues. This further stimulates kinases which are found inside the cells, which immediately phosphorylate adaptor proteins, resulting in the stimulation of various downstream pathways, which include phosphoinositide 3-kinase-protein kinase B, Janus kinase/signal transducers, and phosphoinositide phospholipase C. FGFR1-4 alterations have been found in a wide range of tumour. Fibroblast growth factor receptor-2 fusion is mainly enriched in intrahepatic cholangiocarcinoma and occurs in 5–15 % of cases. Overall, most of the larger intrahepatic cholangiocarcinoma databases have identified 63 different fusing unions along with an association. This fusing frequently joins the N-terminal of FGFR2, which has an intact intracellular tyrosine kinase region, with the C-terminal of polypeptides which has an oligomerization region. The FGFR2 fusion proteins are dimerized by this oligomerization domain in ligand-independent criteria, resulting in continuous downward activation[9].

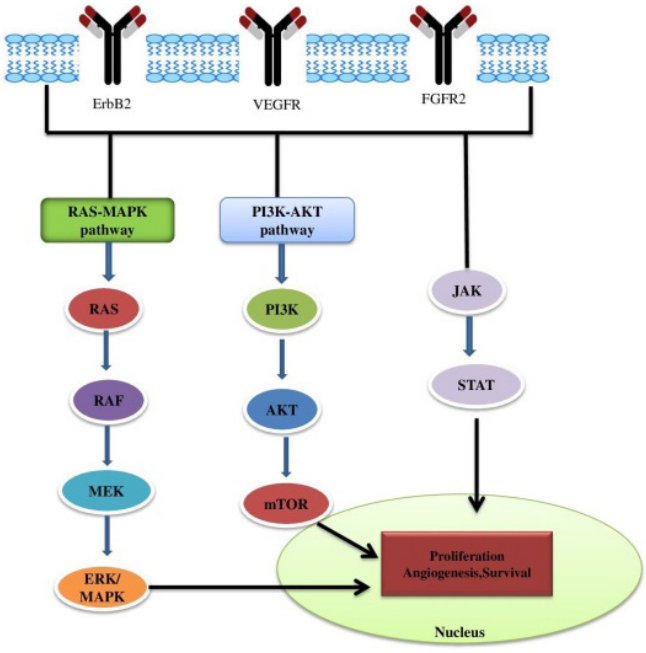

Figure 3. Signaling pathway in CCA.

FGFRs are made up of three regions: an intracellular, an extracellular, and a transmembrane. The extracellular ligands interaction region has three antibody Ig-like regions (Ig-I, Ig-II, and Ig-III) and also a particular region for interacting with the FGFs, histidine, HS ceramide, and different ECM compounds[55]. The intracellular region, having a C-terminal tip and also two separated tyrosine kinases 1 and 2, binds to the cytoplasm region and transmits intracellular FGFR signals. The interaction with the native region of the fibroblast growth factor is necessary for fibroblast growth factor receptor-2 stimulation, causing dimerization by binding site with autophosphorylation in the C-terminal region of the intracellular domain. Four of the FGFRs exhibit structural similarity to other tyrosine kinase receptors, such as Platelet-Derived Growth Factor Receptor (PDGFR) and vascular endothelial growth factor receptor, which have significance for pharmacological treatment[56].

FGFR signalling is one of the most commonly dysfunctional channels in cancer due to numerous types of biological pathways, such as growth and hybridization in the FGFR transcript. The dysfunction of FGFR signalling is used to enhance the development, viability, and growth of anti-cancer chemoresistance as well as the promotion of antibody resistance in tumour cells. In iCCA, it has been observed that FGFR 1–4 upregulation caused by mutations and amplification provides a crucial carcinogenic enhancement. When FGFR4 is activated, it is capable of inducing the development, invasion, and epithelial-mesenchymal

transition of tumour cells, whereas increased secretion of FGFR4 is linked with poor prediction and CCA development. Nevertheless, FGFR2 fusions and translocations represent the most significant FGFR abnormalities in CCA, according to both diagnostic and medical perspectives[57].

According to multiple studies, these fibroblast growth factor receptor-2 molecular translocations are present in 15% to 20% of instances, almost entirely in intrahepatic cholangiocarcinoma fragments, and are generally incompatible with another carcinogenic variant. The FGFR2-BICC1 fusion resulting from t(10;10) (q21;q26), is capable of activating and implicated in the regulation of mitogen-activated protein kinase and the Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit (PI3CA)/ mammalian target of rapamycin pathway, In iCCA, chromosome transfer of FGFR2 leads to the development of carcinogenic target proteins that contain an intact tyrosine kinase region that is attached to a partner protein's C-terminal region with a significant capacity for dimerization and oligomerization. Due to the stimulation of certain molecular pathways, contribute to the beginning of several biomolecular processes, which include the movement, anchoring, and collection of the cancer cell that produces the cancer mass[58].

3.3.2 Epidermal Growth Factor Receptor (EFGR)

The erythroblasts leukaemia viral oncogene homologue group having Receptor Tyrosine Kinase (RTK), EFGR, also called ERBB1, ERBB2, ERBB3 and ERBB4, by complete genetic and bioinformatics methods, has been found in many tumour patients. Erythroblastic Oncogene B (ErBbs) and EFGR have been connected to the development of different kinds of cancer by stimulating cell development, growth, movement, angiogenic processes, tumour growth, and malignant transformation by interacting with the receptor involving EGF, Heparin-Binding Epidermal Growth Factor (HB-EGF), TGF-α, and β-cellulin as high-capacity capability ligands, whereas amphiregulin, epiregulin. In addition, CCA cells have high levels of HB-EGF and amphiregulin (AREG), which function as EFGR ligands and dramatically increase cancer cell viability by activating the EFGR and Extracellular Signal-Regulated Kinase (ERK) pathways[59].

Casitas B-lineage lymphoma (c-Cbl) mediated pinocytosis, destruction of the epidermal growth factor receptor is initially linked with the main downward regulation mechanism of the epidermal growth factor receptor by the generation of the c-Cbl/epidermal growth factor receptor complex. EFGR signalling is sustained because the association of EGFR with c-Cbl is restricting, whereas c-Cbl triggers EGFR disintegration. The stimulation of EGFR accelerates the advancement of CCA through its oncogene pathway and receptors responsible for the growth and development of cancer. EGFR stimulation also participates in tumour development and the chemotherapeutic agents of cancer by the Epithelial-Mesenchymal Transition (EMT), thus increasing the erythroid cells through the induction of the MAPK/ERK kinase, Janus kinase 2, and signal transducer and activator of transcription 3 pathways. E-cadherin is located in the cytoplasm of patients with CCA relative to localization at the cell membrane of biliary tract epithelial cells, and placement is strongly linked with EGFR interpretation in sick people with CCA, so that loss of EGFR activity results in the renovation of cellular articulation of E-cadherin. EGF-initiated EGFR stimulation in CCA cells initiates EMT by decreasing E-cadherin and enhancing like N-cadherin and -spinal muscular atrophy via the initiation of EMT-transcription factors involving Slug and Zinc Finger E-Box Binding Homeobox 1 (Zeb 1). Nevertheless, gefitinib therapy elevates CCA cells' capacity for metastatic spread by EGF/EGFR-induced EMT. Along with this, there is one more pathway that involves EGFR-initiated growth of CCA, which aids in the stimulation of mesenchymal-epithelial transition (c-Met), and Receptor Tyrosine Kinases (RTK) for Hepatocyte Growth Factor (HGF), which helps in the progression of the tumour. The overexpression of the epidermal growth factor receptor decreases survival in individuals with ICC and ECC and is directly related to the upregulation of c-Met. The frequency of ERBB2 production and the advancement of CCA cells by ERBB2-mediated stimulation of the AKT/70-kDa ribosomal protein S6 kinase (p70S6K) signalling pathway evaluates CCA cells' invasiveness and development[60]. Tyrphostin AG1517 and Tyrphostin AG879 are two inhibitors of ERBB2, which efficiently prevent the proliferation of CCA cells, while a combined treatment having synergistic activity on the prevention

of CCA cell proliferation via preventing cyclin D1 and stimulating caspase 3 (CPP32)[61].

3.3.3 Vascular Endothelial Growth Factor Receptor

The VEGF family consists of compounds that comprise various isoforms (VEGF-A, B, C, D, and E) and amnion proliferation factor, which is released via numerous cells, including platelets, neutrophils, and urothelium. The separate role of every subunit of vascular endothelial growth factor is initiated by the link between the polypeptides and its receptor, the vascular endothelial growth factor receptor[62]. Vascular Endothelial Growth Factor receptor-1 (Flt-1) and vascular Endothelial Growth Factor receptor-2 (Flk-1) are two RTKs mostly involved in ontogenesis and vasculogenesis pathways and VEGFR-3 (Flt-4) is primarily active during lymphogenesis. In various studies, the function of vascular endothelial growth factor in cholangiocarcinoma and its capacity to initiate vascular permeability, cell shift, and vasorelaxation, which help in the vasculogenesis of tumour parenchyma, were identified. According to earlier research by Kawahara *et al.*, which identified that VEGF regulation was decreased in CCA patients in comparison with the control patients, there is no relationship between Vascular Endothelial Growth Factor A (VEGF-A) levels and survivability. In comparison with this data, individuals affected by EH bile duct carcinoma identified generally poor prospects for survivability along with a higher VEGF positivity in comparison to individuals in whom the reason is not identified. Yoshikawa *et al.*, examined the vascular endothelial growth factor level in IH-CCA and extrahepatic cholangiocarcinoma, revealing that their regulation is greater in both tumours in comparison to the control. They also established that a higher level is clinically associated with IH-metastasis. Recently, other writers identified the promotion of cyclooxygenase 2 (COX-2)[63] and vascular endothelial growth factor-C in various steps of cholangiocarcinoma for determining their clinical (based on tumour infiltration) and pathologic (based on cell maturation) involvement in this illness[64]. Cyclooxygenase 2 and VEGF-C's upregulation in the late clinical and pathologic stages of cholangiocarcinoma show their role in tumour cell proliferation and metastasis processes. The regulation of VEGF has been identified and associated with progressed disease stages and negative stages. The ability to use an antiangiogenic drug in conjunction with chemotherapeutics is highlighted by the activation of the angiogenic pathway[63].

Bevacizumab[65], a synthetic humanistic monoclonal antibody against VEGF, is a crucial therapeutic tool for cancer[66]. An innovative strategy to increase survival is the standardisation of cancer vasculature. In non-comparative phase II research, an association of bevacizumab with gemcitabine and oxaliplatin (GEMOX) has a seven-month median with acceptable toxicity in metastasis biliary tract cancer. *In vitro* research indicates a time-dependent enhancement of vascular endothelial growth factor level in response to paracrine or autocrine tumour growth factor-β activation, revealing that TGF-β is activated by the transcription factor specific protein 1 (Sp1). Along with this, the traditional target gene, vascular endothelial growth factor, shows the first proangiogenic factor controlled via tumour growth factor-β1 by specificity protein[63].

4. Alkaloids

Alkaloids belonging to a broad category of substances with cyclic structures include one basic nitrogen atom. Alkaloids are widely distributed throughout the plant kingdom and are mostly found in plants belonging to the Leguminosae, Menispermaceae, and Ranunculaceae families[67]. These are some of the alkaloids that are used in cholangiocarcinoma:

4.1 Solamargine

It is an anabolic steroid alkaloid that originated in Solanum species fruit, including *S. nigrum*[68]. The IUPAC name of solamargine is 26-O-beta-D-Glycopyranosyl-22-hydroxyfurost-5-ene-3beta, 26-diol-3-O-beta-diglucorhamnoside, having the chemical formula $C_{45}H_{73}NO_{15}$. Solamargine consists of two components: purapuridine (an oxaza-spiro steroid genin) and CHEBI:30957 alpha-L-rhamnopyranosyl-(1->2)-beta-D-glucopyranose which is connected inside the 3-OH group of purapuridine in the C3 position[69,70]. It may also prevent the growth and promote the apoptosis of various types of malignancies. Apoptosis is a programmable cellular death that has the potential to

act as an anticancer target. Initiation of cell death in the TM may also prevent immoderate cell growth and have a powerful curative effect on cancer[71].

Solamargine treatment of QBC939 cells may cause cell shrinkage, distortion, and programmed cell bodies. It also prevented QBC939 cell proliferation in a dose-dependent way, according to an MTT experiment, with an IC_{50} of 9.81 mM. Whereas changes in the MMP are an early event of caspase-mediated cell death, causing the discharge of hemoprotein in the cell membrane, which could start the caspase-9 enzyme activation. Caspase-mediated cell death is correlated with various proapoptotic individual polypeptides, including Bcl-2-associated X protein (Bax) and caspase 3, and programmed cell death, which has Bcl-2, an X-linked inhibitor of apoptosis and is evaluated via the level of pro-apoptotic to programmed cell death. Thus, it was shown that solamargine enhances the regulation of Bcl-2-associated X-protein, CPP32, cleaved caspase 3, caspase 7, and poly (ADP-ribose) polymerase while reducing the regulation of B-cell lymphoma 2, B-cell lymphoma-extra large, and X-linked inhibitor of programmed cell death protein. Consequently, it can also significantly initiate the pro-apoptosis of human CCA cells. Solamargine-initiated programmed cell death and changes in programmed cell death-related genetic code and polypeptide regulation were identified by applying real-time PCR and ELISA tests. Therefore, by triggering planned cell death and also having an impact on the proteins connected to programmed cell death by inhibiting QBC939 cells' ability to proliferate, solamargine may be effective in the therapy of CCA[72].

4.2 Cepharanthine

Cepharanthine[73] shown in Figure 4 (CEP) is a biscoclaurine, called 12-methoxycarnosic acid cepharanoline, belonging to a significant class of similar in structure (BBIQ) cycled alkaloid, such as limacine, hernandesine, berberine, and trilobamin[74]. CEP is extracted from the flora of the species Stephania, one of the largest species in the Menispermum family, which includes the yellow parilla. The term Stephania originated from the Hellenic word Stefanos, which means "crown" and "garlands". CEP is present in many *Stephania genera*, mainly *S. rotunda Lour* and *S. cephalantha Hayata*, *S. sasakii Hayata*, *S. suberosa Forman*, and *S. epigigaea*[75]. The first documented use of this plant was in 1914 by Bunzo Hayata, a biologist at Taipei Imperial University.

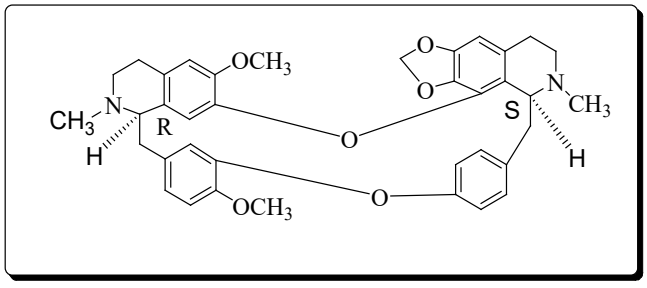

Figure 4. Structure of cepharanthine.

CEP efficiently inhibits nuclear factor kappa-light-chain-enhancer of activated B cells (NF-jB) activity, preventing growth both *in vitro* and *in vivo*. CEP has effective pro-apoptotic activity on cholangiocarcinoma cells to start the process of apoptosis and decrease cancer proliferation in non-occlusive disease/Scid/Janus kinase-3-deficient mice lacking adverse reactions. Cepharanthine prevented cell viability of the cholangiocarcinoma cell line KKU-213, and KKU-M214, at therapeutically acceptable doses of 2.5 to 20 mg/mL (4.12-32.96 IM), indicating its clinical effectiveness for CCA[76]. CEP decreases cancer viability by initiating programmed cell death in cholangiocarcinoma cells, as proven via deoxyribonucleic acid cascade organization, a defining characteristic of programmed cell death. CEP-initiated apoptosis in cholangiocarcinoma cells via the stimulation of CPP32/Yama/apopain and caspase 9 (CASP9), two of these enzymes having a significant role in programmed cell death regarding the stimulus. At high dosages, CEP may produce an excess amount of ROS in tumour cells, which then directly affects the cell membrane, sealing CYC and stimulating CASP9 and CPP32. By preventing NF-jB stimulation, CEP reduces NF-jB activity, resulting in the inhibition of NF-jB nuclear translocation and deoxyribonucleic interaction properties[77]. Thus, it is significantly used in the treatment of CCA.

4.3 Piperlongumine

A well-known natural bioactive alkaloid and amide called piperlongumine[78] (PL), also known as piplartine, is present in the radicle of the herb *Piper longum* (pipali). Additionally, it is present in many of the Word Hippo The saurus species, including *Piper alatabaccum Trel,* Yunck, *Piper chaba Hunter,*

Piper cenocladum C, and *Piper tuberculum Benth*. The IUPAC of PL is 1-[3-(3,4,5-Trimethoxy-phenyl)-acroyl]-5,6-dihydro-1H-pyridin-2-one. PL has the chemical formula $C_{17}H_{19}NO_5$, which has a molar mass of 317.34 g/mol[79]. The structure of Piperlongumine is shown in Figure 5.

The activity of PL on individual cholangiocarcinoma cell lines was evaluated by the cell growth test by applying the SRB assay to five individual cholangiocarcinoma cell lines, including KKU-055, KKU-100, KKU-139, KKU-213, and KKU-214, and two depicted cell lines, including MMNK-1 and NIH3T3, after being exposed to various concentrations of PL (0, 2.5, 5, 10, and 15 M) for 48 h. The most responsive cell line was KKU055, while the least sensitive were KKU-100 and NIH3T3. For the cholangiocarcinoma cell lines KKU-055 and KKU-213, KKU-214, KKU-139, and KKU-100, the corresponding IC_{50} of piperlongumine via applying the CompuSyn software were 4.2, 5.2, 6.2, 8.8, and 15.9 M, and 5.7 and 12.7 M for MMNK1 and NIH3T3. To enhance the precision of the IC_{50} value of KKU-100 and NIH3T3, a value of 0 to 100 M was determined to increase the cell viability of KKU-100 and NIH3T3. In KKU-100 and NIH2T3, the IC_{50} values for PL were 10.6 and 8.6, respectively. Western blot tests demonstrated the stimulation of caspase3 and BAX in KKU-055. Thus, piperlongumine is effectively used in the treatment of cholangiocarcinoma[80].

4.4 Mahanine

Mahanine, known as 3,5-dimethyl-3-(4-methylpent-3-enyl)-11H-pyrano[3,2-a]carbazol-9-ol is a carbazole alkaloids present in the succulent part of the Thai edible Micromelum mintum, where it is typical to develop CCA linked to the liver fluke. Additionally, this compound is also present in certain related species and the curry leaf plant *Murraya koenigii*. These algae are also found in Southeast Asia and the South[81].

In cholangiocytes, the regulation of Microphthalmia-Associated Transcription Factor (MITF) is kept at an immunohistochemically undetected level. Microphthalmia-Associated Transcription Factor (MITF), having a bHLH-LZ framework, was identified as a crucial stimulator for melanocyte and RPE growth. Additionally, further researchers identified the pleiotropic roles of MITF in many organs, such as the kidney, lung, olfactory bulb, and frontal brain. To regulate the stimulation of MITF activation in the liver, immunohistochemically analyze the regulation of MITF by applying CCA specimens of the humanistic hepatic malignancy tissue array. MITF immunoactivity was identified in CCA groups (2/7 specimens; 29%). Additionally, 86% of CCA patients showed antibodies for glioma-associated oncogene transcription factor-1, a transcriptional factor of the Hh pathways. The activities of mahanine were identified as HuCCT1 and KKU-100 in individual CCA cells. By employing Western blot analysis, it was discovered that mahanine (25 M) possesses potentinal cytotoxicity in the liver malignancy cell line, which is connected to higher levels of MITF. The presence of measurable MITF antibody reactivity is connected with poor survival in people with cholangiocarcinoma because MITF is substantially expressed in subsets of the disease. The equivalence between Hh signalling and cellular stressors may control the level of MITF regulation in liver malignancy. Thus, it is significantly used in the treatment of CCA[82].

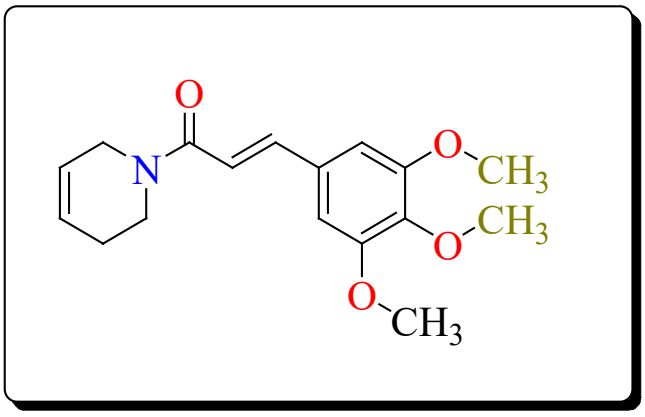

Figure 5. Piperlongumine.

4.5 Berberine

Berberine shown in Figure 6, a leucoline alkaloid, is isolated from various Chinese medicine herbs, including *Oregon hollygrape, European barberry* and *Berberis aristate*. The IUPAC name of 8-Hydroxy-7,8-dihydroberberine. It is yellow in colour and bears nitrogen isoquinoline alkaloid, which has been extracted from the rhizome, seed, branch, blossom, and sour fruitage of *Chinese goldthread*, *Warneris canadensis* Mill, *Berberidaceae* spp, and common barberry[83]. Berberine dramatically reduced the

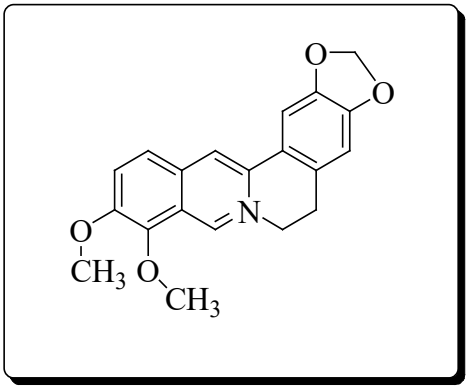

Figure 6. Berberine.

growth of CCA cells by preventing the action of the extracellular signal-regulated kinase 1/2, nuclear factor kappa B, signal transducer, and activator of transcription 3 pathways. Berberine affects signal transducer and activator of transcription 3 (STAT3), a significant mechanism which plays a significant role in the aetiology of numerous malignancies involving CCA. The current investigation showed that berberine decreased STAT3 expression and phosphorylation at Tyr705 and Ser727. Nuclear factor kappa B (NF-κB), a transcriptional factor having a vital function in the regulation of numerous genes, including in the progression of malignancy is dramatically activated in various cancer cells, such as CCA. Prevention of NF-kB dramatically decreased cancer cell proliferation, and motility, triggering programmed cell death. In several different ways, berberine can suppress NF-kB. Berberine has anti-tumour action against two cholangiocarcinoma cell lines, such as KKU-213, and KKU-214. It also reduced the regulation and stimulation of signal transducer and activator of transcription 3 and extracellular signal-regulated kinase in both cell lines along with a compatible condition; thus, berberine has the strongest action on the regulation, stimulation of NF- kB in KKU-213 as compared to KKU-214. Because the KKU-213 is further reliant on NF-kB action as compared to the KKU-214. KKU-213 has a greater baseline concentration of NF-kB intermediates (p100 and p105) as compared to KKU-214 cells. When NF-kB activation was prevented, KKU-213 was impacted more severely than KKU-214. This assumption is correlated with the help of dehydroxymethylepoxyquinomicin (DHMEQ), a particular NF-κB inhibitor, having a higher impact on KKU-213 than KKU-214 *in vitro* and *in vivo*. A second signalling mechanism other than NF-kB may have been used by Berberine to alter KKU-214. Recently, it was identified that berberine may suppress the regulation of several genes, including cyclooxygenase-2 and prostaglandin 2 (PGE2), MicroRNA 93, Phosphatase and tensin homolog, Akt, and EGFR. These also affect cell growth and possibly play a role in the action of berberine in KKU-M214. The enhanced NF-kB regulation in the control cell at 48h in comparison to the at 24h is shown in both CCA cell lines. That finding was most likely caused by the CCA cell's activation of IL-6. Enhancing the regulation of IL-6 may also promote the stimulation of NF-kB in cancer cells because the culture media of the CCA cell lines included a significant quantity of physiologically active IL-6[84].

The prevention of signal transducer and activator of transcription 3 and nuclear factor kappa B pathways in cholangiocarcinoma lines may be because of the stimulation of extracellular signal-regulated kinase 1/2 via reducing extracellular signal-regulated kinase 1/2 regulation. Extracellular Signal-Regulated Kinase (ERK) stimulation stimulates NF- kB signalling[85]. In contrast, PD98059, an inhibitor of ERK, specifically reduced the NF-kB pathway. STAT3 is phosphorylated on the serine 727 sites of STAT340 by ERK, while STAT3's tyrosine 705 residue is phosphorylated by JAKs and Src kinases. Numerous studies have identified that STAT3 must be phosphorylated at both positions to be fully activated. As a result, the suppression of ERK1/2 activation and phosphorylation contributed to the prevention of nuclear factor kappa and signal transducer and activator of transcription 3 phosphorylation. Suppression of ERK phosphorylation may result in decreased regulation and phosphorylation of nuclear factor kappa B signalling (p50, p52, p65) in KKU-213, and KKU-214 cells treated with PD98059[84]. Thus, it is significantly used in the treatment of CCA.

4.6 Tiliacorinine

Tilliacorinine is a bisbenzlisoquinoline alkaloid that was discovered in the *Tiliacora triandra* (*Colebr*) plant, a tropical medicinal herb, and has a molecular weight of 576.26[86]. It also decreased cancer proliferation in xenografted mouse models with CCA and successfully prevented cholangiocarcinoma cell proliferation by promoting programmed cell death. The proliferation

suppression activity of tiliacorinine on individual cholangiocarcinoma cells (KKU-100, KKU-M213, and KKU-M214) was assessed using the SRB test. M055 with IC_{50} values of 4.5+0.3, 5.7+0.2, 6.1+0.3, and 7.0+0.6, respectively, tiliacorinine had growth-inhibitory effects on the examined cells in the following order: KKU055, KKU-M213, KKU-M214 and KKU-100. It was discovered that tiliacorinine causes apoptosis by activating caspase-3, 9, which leads to Poly (ADP-ribose) polymerases (PARP) cleavage. Tiliacroinine promotes programmed cell death by caspase stimulation, KKU-M214 was treated with the IC_{50} (6M) of tiliacorininie for 24, 48, and 72 h, and whole cell lysates were then submitted to western blotting. Tiliacorininie stimulated Cysteine-Aspartic Acid Protease (CASP3), and poly (ADP-ribose) polymerase cleavages in a time-dependent manner. In this study, tiliacorinine had an apoptotic impact on human CCA cells and reduced tumour proliferation in CCA xenografted mice, demonstrating that auspicious alkaloids are a potent therapy for CCA and promoting the expression of antiapoptotic proteins, X-linked inhibitor of apoptosis protein (XIAP) and BcIxL, in a time-dependent manner. Tiliacorinine may be an effective therapy for CCA treatment[87]. The structure of Tilliacorinine is shown in Figure 7.

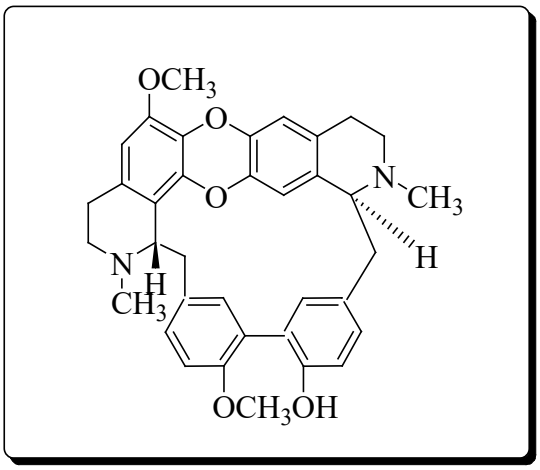

Figure 7. Tiliacorinine.

4.7 Evodiamine

Evodiamine in Figure 8[88] is a quinolone alkaloid that is extracted from euodia ruticarpa (*T. ruticarpum*)[89]. Two CCA cell lines were examined to determine how evodiamine affected the growth of cholangiocarcinoma

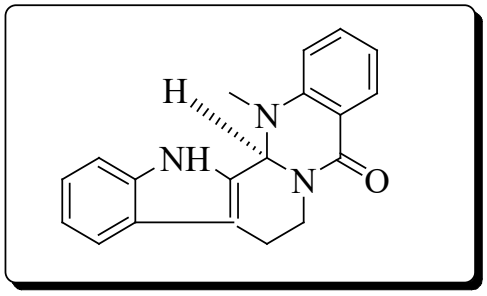

Figure 8. Evodiamine.

cells, human cholangiocellular carcinoma cell line (HuCCT-1) and TFK-1, was studied including evodiamine at various doses (0, 5, 10, 20 or 40 μM) over various periods (24, 48, 72 h), were assessed using the *Cell Counting Kit-8 (*CCK-8) assay. According to the results, evodiamine reduced TFK-1 and HuCCT-1 cell viability in a time- and dose-dependent manner. To identify the long-time proliferation-suppressing activity of evodiamine, we incubated HuCCT-1 and TFK-1 cells with evodiamine (0,20 or 40 μM) for 24 h, washed the cell then cultivated them for an additional 14 days in growth medium. Nuclear chromatin condensation and fragmented punctuate blue nuclear fluorescence were observed in TFK-1 cells. The result identified that evodiamine may activate CCA cancer programmed cell death. The stimulation of cysteine-aspartic acid protease (CASP3) and cysteine-aspartic acid protease (CASP9) are two important apoptosis pathways. According to the findings of the current investigation, the phosphorylation at Tyr705 rather than the Ser727-phosphorylated site was responsible for the inhibiting impact of evodiamine. This inhibitory effect shows that evodiamine blocks the phosphorylation of signal transducer and activator of transcription 3 at the Tyr705 site[90]. Evodiamine may be an effective therapy for CCA treatment.

4.8 Matrine

An alkaloid known as matrine[91] was found in the common herb *Sophora flavescens* Ait[92]. Matrine treatment helps decrease the level of phosphorylation of the signal transducer and activator of transcription 3 (STAT3), Tyr 705 in comparison to a control cell. Additionally, marine treatment dramatically decreased the phosphorylation of Janus kinase 2 (Tyr1007/1008). Luciferase reporter analysis showed that signal

transducer and activator of transcription 3 properties were inhibited via matrine treatment of CCA cells. It also inhibited the JAK2/Signal Transducer and Activator of transcription 3-dependent transcription properties in CCA cells. It also helps in decreasing the stimulation of Mcl-1 in the CCA cells. Thus, matrine may be an effective therapy for CCA treatment[93]. The structure of the matrine is shown in Figure 9.

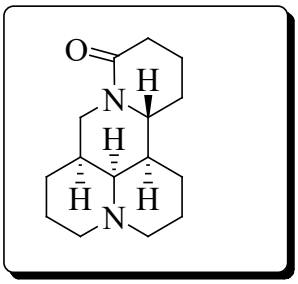

Figure 9. Matrine.

5. Conclusion

CCAs are extremely aggressive biliary tumours with poor prognoses that exhibit both intra- and intra-tumor heterogeneity. Cirrhosis, bile duct stones, Caroli disease, Primary Sclerosing Cholangitis (PSC), bacterial infections, hepatitis liver flukes, types B hepatitis and viral hepatitis C (HCV) have all been identified as significant risk factors, but they are only present in a small proportion of individuals. Moderate risk for CCA is linked to other, more common variables. Operation, diffraction, locoregional therapy, and chemotherapy are insufficient for the treatment of cholangiocarcinoma. There has been a rise in recent years, in research into the signalling pathways and aetiology of these tumours to develop more effective treatments. Since it was discovered that alkaloids are more potent and effective against cholangiocarcinoma cell lines, they have gained more significance in the treatment of cholangiocarcinoma. Cholangiocarcinoma cell lines' capacity to proliferate, migrate, and invade is constrained by their promotion of apoptosis. In this review, we highlight advancement in the study of cholangiocarcinoma, with an aim on the signalling pathway's mechanisms and effective natural alkaloids that have powerful anti-cholangiocarcinoma activity. These findings will help researchers quickly develop efficient and feasible pharmaceutical compounds.

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