ABSTRACT
Nowadays cancer has emerged as a leading cause of death. Therefore, the major research is aimed at discovering the drugs which will be more potent and efficacious for the treatment of cancer. For this we have considered Noscapine which has been discovered as safe antitussive agents and binds to tubulin, arrests dividing cells in mitosis and induces apoptosis. Noscapine is a Phthalideisoquinoline alkaloid obtained from Opium latex. Unlike other opium alkaloids, noscapine is non sedative and has been used as antitussive drug in the Pediatric preparation in various countries. Its binding to tubulin and further disruption of the spindle in the mitosis process; it has been introduced as an anti-mitotic agent. Many other antimiotic compounds such as paclitaxel and vinblastine are also being used clinically in the treatment of different cancers but all are associated with side effects and have multiple drug resistance. On the other hand it has been reported that noscapine has minimal side effects. Therefore, noscapine and its analogs have great potential as novel anticancer agents.

Keywords: Noscapine, antimitotic, microtubules, tubulin.

INTRODUCTION
Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries. The burden of cancer is increasing in economically developing countries as a result of population aging and growth as well as, increasingly, an adoption of cancer-associated lifestyle choices including smoking, physical inactivity, and “westernized” diets. (1)

About 12.7 million cancer cases and 7.6 million cancer deaths are estimated to have occurred in 2008 worldwide with 56% and 64% of the deaths in the economically developed &
developing countries respectively. Breast cancer in females and lung cancer in males are the most frequently diagnosed cancers and the leading cause of cancer death for each sex in both economically developed and developing countries, except lung cancer is preceded by prostate cancer as the most frequent cancer among males in economically developed countries. These cancers were followed, without specific rank order, by stomach and liver cancers in males and cervix and lung cancers in females in economically developing countries and by colorectal and lung cancers in females and colorectal and lung or prostate cancers in males in the economically developed world. (2)

Cancer is not just one disease but a group of more than 100 diseases that can affect any part of the body. Mortality rates for all cancers combined in developed countries are only 21% higher in males and only 2% higher in females. Such disparities in incidence and mortality patterns between developed and developing countries will reflect, for a given cancer, regional differences in the prevalence and distribution of the major risk factors, detection practices, and/or the availability and use of treatment services. There are many different types of cancer which cause a large number of mortality worldwide such as female breast cancer, colorectal cancer, lung cancer, prostate cancer, liver cancer and cervical cancer. (2, 4)

Table I – Types of Cancer & Risk Factor

<table>
<thead>
<tr>
<th>Types of Cancer</th>
<th>Estimated cases</th>
<th>Estimated death</th>
<th>Risk factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female Breast Cancer</td>
<td>1.58 million</td>
<td>458,400</td>
<td>Long menstrual history, null parity, uses of post menopausal hormone therapy or oral contraceptives, late age at first birth &amp; alcohol consumption.</td>
</tr>
<tr>
<td>Colorectal Cancer</td>
<td>0.8 million</td>
<td>408,700</td>
<td>Smoking, physical inactivity, overweight, obesity, red &amp; processed meat consumption &amp; excessive alcohol consumption.</td>
</tr>
<tr>
<td>Lung Cancer</td>
<td>1.6 million</td>
<td>1.0 millions</td>
<td>Smoking accounts for 80% of the worldwide lung cancer. Exposure to several occupational &amp; environmental carcinogen.</td>
</tr>
<tr>
<td>Liver Cancer</td>
<td>748,300</td>
<td>695,900</td>
<td>HBV infection</td>
</tr>
<tr>
<td>Cervical Cancer</td>
<td>529,800</td>
<td>275,100</td>
<td>HPV infection</td>
</tr>
</tbody>
</table>

In cancer treatment strategies the main aim is to eradicate the entire population of neoplastic cells & the immune response which contributes substantially to pathogenesis of disease. This
lead to multiple approaches for the treatment of cancer including chemotherapy, radiotherapy and with the help of natural products. Anti-cancer products available in the market are mainly synthetic, semi synthetic & natural anti-tumor products. Most of the synthetic & semi synthetic products have many side effects & failures in the treatment after a period due to multidrug resistance. (5) In current scenario, more than 65-70% anti-cancerous agents are derived from natural sources, including plants, marine organisms & microorganism.

On cellular level or on the basis of mechanism of action the anticancer drugs can be classified into tubulin binding, DNA binding and DNA damaging. The anticancer drugs belonging to the antibiotic category plays an important role in the treatment of various cancers. These antimitotic drugs can be further classified into drugs which polymerize the tubulin and drugs which depolymerize the tubulin. (6)

**Microtubule as target for cancer chemotherapy**

Microtubules are one of the major components of the cytoskeleton which are essential for many cellular processes including maintenance of cell structure, protein transportation and mitosis. These are also referred to as conveyer belts inside the cell.

The microtubules are composed of a group of cylindrical proteins know as tubulins and perform many of their functions by binding to MAPs i.e. microtubule associated proteins. Microtubules are directly involved in the formation of mitotic spindle which helps in segregating the replicated chromosomes towards two daughter nuclei at the end of mitosis. Involvement of microtubules in this particular cell cycle event makes them an important target in cancer chemotherapy. (7)

During the cell division i.e. mitosis, microtubules play an important role in segregation of chromosomes via spindle formation. Microtubules as the name suggests are the hollow tube like structures with a diameter of 15-25 nanometer and form the major part of the cytoskeleton. Their length may vary from 200 nm to 25 micrometers. This hollow structure is formed by an imperfect helix like arrangement of the protofilaments. The protofilaments in turn, are the product of end to end polymerization of the tubulin heterodimers namely alpha tubulin and beta tubulin.

This leads to the formation of protofilaments with beta subunits exposed at one end and alpha subunit at the other. These are designated as plus (+) and minus (–) ends respectively. In a microtubule the protofilaments bundle parallel to each other so that there is one end with beta
tubulin subunit (plus end) exposed and the other with alpha (minus end) unit exposed. The minus end is capped, so that elongation occurs from the plus end. (8)

The mitotic spindles are formed by attachment of GTP-tubulin to the growing end of the protofilament. The microtubules undergo rapid assembly (rescue) and disassembly (catastrophe) leading to their dynamic instability. This dynamic instability along with their involvement in mitotic spindle formation helps in the metaphase to anaphase transition of the mitosis. This continued assembly and disassembly process in microtubules are crucial to the normal cell division and any interference in this leads to cell death via apoptosis. Usually the anti-mitotic agents arrest the metaphase to anaphase transition in mitosis. The defective spindles formed due to disturbances in dynamics of microtubules at low concentrations of the anti-mitotic agent are unable to cross the mitotic spindle checkpoint and initiate the anaphase stage. This leads to prolonged mitotic arrest and finally cell death by apoptosis. (9)

In addition to the dynamic instability, microtubules have another kind of dynamic behaviour, called treadmilling, which is net growth at one microtubule end and balanced net shortening at the opposite end. In involves the intrinsic flow of tubulin subunits from the plus end of the microtubule to the minus end and is created by differences in the critical subunit concentrations at the opposite microtubule ends. (11)

**Microtubule Targeting Drugs**

As microtubules play a crucial role in cell division it makes them a very suitable target for the development of chemotherapeutic drugs against the rapidly dividing cancer cells. The effectiveness of microtubule targeting drugs has been confirmed by the successful use of several vinca alkaloids and taxanes for the treatment of a wide variety of human cancers. Their clinical success has prompted a worldwide search for compound with similar mechanisms of action but having improved characteristics. This search has resulted in the discovery of a number of novel microtubule targeting drugs, the majority of which are natural products. Their natural sources and chemical structures are remarkably diverse, making microtubules the only target for which such a diverse group of anti-cancer agents has been identified. Microtubule targeting agents are divided into two traditional categories:

a) Microtubule destabilizing agents such as the vinca alkaloids (vinblastine, vincristine, etc.) colchicines
b) Microtubule stabilizing agents such as the taxanes (paclitaxel and docetaxel)

**Microtubule destabilizing agents**

**Vinca alkaloids**

The vinca alkaloids vinblastine and vincristine were the first natural products to enter in the clinical use for cancer chemotherapy. These compounds were isolated by two different research groups in late 1950’s and early 1960’s from Madagascar periwinkle known as *Vinca rosea* or *Catharanthus roseus*.

The vinca groups of alkaloids binding to the beta subunit of tubulin are constituted by several closely related compounds. These include vincristine, vindesine, vinorelbine and vinflumine, which are the semisynthetic vincaalkaloids. Vinblastine and vincristine are in clinical use as anticancer drugs.

![Chemical structure of vincristine](image)

**Vincristine**

They are also used in combination therapy of acute leukemias and lymphomas, bladder and breast cancers. (14)

**Combretastatins and derivatives**

Although the vinca alkaloids are the only tubulin polymerization inhibitor compounds which are in clinical use, there are several other groups of compounds which bind to same domain of the tubulin and have similar mechanism of action. Many of these analogs are in advanced stages of clinical trials e.g. Combretastatins, which were isolated from the root bark of *Combretum caffrum*. They are well-known as antimitotic agents and Combretastatin A2 (CA2) & Combretastatin A4 (CA4) are the most potent members of this family.

CA4 is highly cytotoxic than its tubulin destabilizing activity. This compound is in phase III trials for treatment of cervical, colorectal, NSCLC, prostate and ovarian cancers. (15)
Hemiasterlin

Hemiasterlin is a tripeptide of marine origin. It was first isolated from *Hemiasterella minor* and found to be active against murine leukemia cell lines. Later, its antitubulin and antimitotic activity was discovered by Anderson. The phenyl alanine derivative of the parent compound, HTI-286 has been found to be more potent and more synthetically accessible. Both these molecules are in clinical trials. \(^{(16)}\)

Rhizoxin

Rhizoxin was isolated from a plant pathogenic fungus *Rhizopus chinensis* and was discovered to be inhibitor of tubulin polymerization. It is a macrocyclic lactone, although very similar to maytansine, it is comparatively more potent against human tumor cells. It has been synthesized and gone through the clinical trials. The molecule is yet to be approved for clinical use. \(^{(17)}\)

Microtubule stabilizing agents

The taxanes

Paclitaxel has been the main chemotherapeutic agent for the various types of cancers including breast, ovarian and the prostate cancer. This compound was first isolated and reported from the pacific yew tree bark in 1960 and named as Taxol. The new and currently used generic name Paclitaxel was given when the drug was developed commercially by Bristol-Mayers Squibb and sold under the trade name Taxol.

The success of the paclitaxel led to the development of many of its analogs which are currently in clinical trials. The only analog approved in USA is the Docetaxel, which is a semi-synthetic analog and was developed in France. \(^{(12)}\)
Epothilones

Epothilones belong to macrolide class of the drugs and act as microtubule stabilizers. They are produced by Mycobacterium *Sorangium cellulosum* and initially found to have antifungal and cytotoxic activity.

Later, the cytotoxic activity of these epothilones A and B was found to be associated with mitotic arrest, which occurs *via* over polymerization of microtubules.\(^{(13)}\)

**Table II. Diverse origin of Some Natural Products.**\(^{(8)}\)

<table>
<thead>
<tr>
<th>ORIGIN</th>
<th>DRUG</th>
<th>SOURCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLANT</td>
<td>Paclitaxel</td>
<td><em>Taxus brevifolia</em> (Yew tree bark)</td>
</tr>
<tr>
<td></td>
<td>Docetaxel</td>
<td><em>Taxus baccata</em> (semi-synthetic)</td>
</tr>
<tr>
<td></td>
<td>10-deacetylbaclatin III</td>
<td><em>Taxus brevifolia</em> (Yew tree leaves)</td>
</tr>
<tr>
<td></td>
<td>Vinblastine</td>
<td><em>Vinca alkaloids</em></td>
</tr>
<tr>
<td></td>
<td>Vincristine</td>
<td></td>
</tr>
<tr>
<td>BACTERIAL</td>
<td>Epothilones</td>
<td><em>Sporangium cellulosum</em> (myxobacterium)</td>
</tr>
<tr>
<td></td>
<td>Cyclostreptin</td>
<td><em>Streptomyces sp.</em></td>
</tr>
<tr>
<td>FUNGAL</td>
<td>Rhizoxin</td>
<td><em>Rhizopus chinensis</em></td>
</tr>
<tr>
<td></td>
<td>Phomopsin A</td>
<td><em>Phomopsis leptostomiformis</em></td>
</tr>
<tr>
<td></td>
<td>Ustiloxin</td>
<td><em>Ustilaginoidea virens</em></td>
</tr>
<tr>
<td>MARINE</td>
<td>Discodermolide</td>
<td><em>Discoderma dissolute</em> (marine sponge)</td>
</tr>
<tr>
<td></td>
<td>Dictyostatin</td>
<td><em>Spongia</em> (marine sponge)</td>
</tr>
<tr>
<td></td>
<td>Peloruside</td>
<td><em>Mycale hentscheli</em> (marine sponge)</td>
</tr>
<tr>
<td></td>
<td>Dolastatin 10</td>
<td><em>Dolabella auricularia</em></td>
</tr>
<tr>
<td></td>
<td>Dolastatin 15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Halichondrin</td>
<td><em>Halichondria okadai</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Kadota</em></td>
</tr>
</tbody>
</table>
Side Effects Associated with the microtubule stabilized or destabilized drugs

Although the above mentioned drugs are used in treatment of wide range of different cancers but these causes neuropathy. Peripheral neuropathy has been a limiting factor in the development of several agents, for e.g. Cryptophycins, some analogues of taxol & camptothecin. In contrast, there have been few reports of central nervous system (CNS) toxicity with the above agents, partly due to the fact that they are Pgp efflux pump substrates and thus do not cross the blood brain barrier. The development of newer agents which are not substrates of Pgp might be associated with CNS toxicity, or with activity against tumors within the CNS. Neuropathy is particularly important when considering the combination of these agents with other potential neurotoxic agents. (18)

Myeloid toxicity and neutropenia are the other common side effects associated with the microtubule-targeted agents.

These microtubule binding agents have some mutagenic properties and hence there are risks of secondary tumors. The cells which get exposed to these compounds can develop aneuploidy due to miss aggregation, therefore there is increase the risk of iatrogenic leukemias and/or solid tumors. (19)

Thus, there is still a need for effective drugs with minimal toxic effects, improved solubility & one that can evade the difficulty of development of resistance.

In the search for more safe and efficacious drugs, we chose to study some of the naturally occurring alkaloids, in specific, the family of opium alkaloids. Opium alkaloids clinically used as analgesics, antimalarial, antispasmodics & in the treatment of tumors. These properties of opium alkaloids have made them potent pharmacological agents. There are at-least 25 active chemicals called opiates that can be extracted from opium. These fall into two general categories:
1. Phenanthrene alkaloids i.e. morphine & codeine, are used as analgesics & cough suppressants.
2. Isoquinoline alkaloids i.e. noscapine (antitussive) & papaverine (an intestinal relaxant) have no significant influence on central nervous system.

Noscapine, a phthalideisoquinoline alkaloid obtained from opium has been discovered to have powerful anti-cancerous properties although its antitussive property and non-toxic nature was discovered in the early 20th century.

Most of the anticancer drugs have limited solubility in aqueous solvents and exhibit narrow therapeutic indices. But the main advantage of noscapine over other antimicrotubule drugs is water solubility and feasibility for oral administration. Therefore we planned to study about noscapine in detailed. (20)

**Noscapine & its chemistry**

Noscapine is a phthalideisoquinoline alkaloid constituting 1-10% of the alkaloid content of opium plant *Papaver somniferum*, has been used as a cough suppressant. The mechanisms for its antitussive action are unknown, although animal studies have suggested central nervous system as a site of action. The lactone ring is unstable and opens in basic media. The opposite reaction is presented in acidic media. The bond C1-C3’ is also unstable. This is the bond connecting the two optically active carbon atoms. When noscapine is reduced with zinc/HCl, the bond C1-C3’ saturates and the molecule dissociates into hydrocotarnine (2-hydroxycotarnine) and meconine (6, 7-dimethoxyisobenzofuran-1(3H)-one). In aqueous solution of sulfuric acid and heating it dissociates into Cotarnine and Opianic acid.

The phthalideisoquinoline alkaloids are oxygentated at C-6, C-7, C-4’ and C-5’ and some are oxygentated at C-8. The two point of asymmetry in these alkaloids are: C-1 of the tetrahydroisoquinoline unit and the C-9 of the phthalide moiety. The two best known examples are hydrastine from *Hydrastis canadensis* L. (Ranunculaceae) and (-)-α-narcotine from the *Papaver somniferum* L. (Papaveraceae). It usually posses a tetra cyclic nucleus incorporating a γ-lactone ring. They differ from each other either in the nature and position of the aromatic substituent or in the stereochemistry at the asymmetric centre at C-1 and C-9. (21)
Noscapine indicating C-9 carbon (1)

**Noscapine as antimitotic agent**

Noscapine binds to the tubulin subunits, alters tubulin assembly, arrests a variety of mammalian cells in mitosis and causes apoptosis in cycling cells. This may also be true for other agents known to cause mitotic arrest. For example, taxol promotes tubulin assembly and inhibits microtubule depolymerization.

The other agents are colchicine analogs and vinblastine, which arrest cells in mitosis. However, the nanomolar concentrations at which these agents can cause mitotic arrest are well below their ability to depolymerize microtubules.

It has thus been suggested that these drugs act by a mechanism of kinetic stabilization. Noscapine bound tubulin subunits can assemble into microtubules and that stoichiometric micromolar concentrations are required to elicit these effects. Although noscapine has chemical moieties that similar to those of colchicine and podophyllotoxin, but they bind to different sites of tubulin. We have said that superficially, noscapine shares similar chemical groups with colchicine and Podophyllotoxin but the stereosstructure of noscapine, colchicine, and podophyllotoxin differ. Therefore, it is possible that noscapine may form other contacts on the surface of tubulin. Although at a lower dose (20 mg/kg), noscapine also showed promising results, at a higher dose (120 mg/kg), the antitumor activity was better. \(^{(22)}\)

The significant *in vivo* antitumor activity coupled with its minimal toxicity is probably derived from the weak interaction between noscapine and tubulin. Noscapine does not bind to tubulin as strongly as colchicine, but its interaction is adequate to arrest mitosis. As a consequence, it may be that the mechanism by which noscapine kills...
cancer cells is a result of this kinetic stabilization of the microtubules and not the action of the drug on the assembly and disassembly of the polymer. Perhaps it is this relatively unique combination of properties that makes noscapine so special. Collectively, these data argue strongly that noscapine and its analogs may be good chemotherapeutic agents for the treatment or clinical management of some types of human cancers.

**Apoptosis by Noscapine**

Noscapine study indicates that it induces apoptosis on two myeloid cell lines, apoptosis-proficient HL60 cells and apoptosis-resistant K562 cells. An increase in the activities of caspase-2, -3, -6, -8 and -9, along with increased poly (ADP ribose) polymerase cleavage, detection of phosphatidylserine on the outer layer of the cell membrane. The induction of apoptosis was associated with activation of the JNK signaling pathway concomitant with inactivation of the ERK signaling pathway and phosphorylation of the antiapoptotic protein Bcl-2. Noscapine-induced apoptosis was associated with the release of mitochondrial protein AIF and/or cytochrome C. In some glioma cell lines, only AIF release occurred without cytochrome C release or PARP cleavage; while in others, AIF release occurred together with cytochrome C release and was associated with PARP cleavage. That suggests the potential importance of noscapine as a novel agent for use in patients with glioblastoma multiforme due to its low toxicity profile and its potent anticancer activity. (23)

**Types of cancers which can be treated by Noscapine**

Noscapine effectively inhibits the progression of various cancer types both in vitro and in vivo with no obvious side effects even if this drug was initially used as a cough suppressant. The types of cancers which can be treated with noscapine and its analogs are:

**Lymphoma:** Growth of T-cell lymphoma was inhibited by noscapine in a dose-dependent manner. Also, noscapine can cause tumor regression when administered in the drinking water. A nitro-analog of noscapine, 9-nitro-noscapine effectively inhibits proliferation of drug-resistant lymphoblastoid cell line, with no effect on the cell cycle of normal human fibroblast cells. (24)

**Breast Cancer:** It has been shown that noscapine can arrest mammalian cells at mitosis stage; Noscapine causes apoptosis by binding to microtubule assembly, and arrests cells in mitosis.

**Ovarian Carcinoma:** Noscapine inhibits the proliferation of both paclitaxel sensitive and paclitaxel-resistant human ovarian carcinoma cells. Noscapine is able to arrest these human
ovarian cells at mitosis. This means that noscapine might bind to tubulin at a site different from that for paclitaxel.

**Colon Cancer**: Although colorectal cancer is relatively resistant to many chemotherapeutic agents, noscapine induces apoptosis in a p53-dependent manner, which needs p21 induction. Therefore, noscapine can cause cell death in colon adenocarcinoma cells expressing both p53 and p21. \(^{(25,26)}\)

**NOSCAPINE ANALOGS**

Several noscapine analogs have been reported which have much better therapeutic indices and improved pharmacological profiles. Noscapine consists of isoquinoline and benzofuranone ring system attached by a labile C-C chiral bond & both rings contain many vulnerable methoxy groups. The synthetic analogs reported till date can be classified into different classes regarding the position of their substitution: \(^{(27)}\)

![Structure of Noscapine](image)

**Table for Different analogs of Noscapine & its activity** \(^{(30,31,32,33,35,36,37)}\)

<table>
<thead>
<tr>
<th>Substituent Position</th>
<th>Change In Substituent</th>
<th>Most Active compound of the group</th>
<th>Activity on Mitotic Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Halogen Group</td>
<td>9-Bromo Noscapine</td>
<td>Arrest G2/M-Phase</td>
</tr>
<tr>
<td></td>
<td>Br,F,Cl,I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>-OH, -COCH3, -COPh, -CONHPh</td>
<td>7-Phenylcarbamatonoscapine</td>
<td>Arrest G2/M Phase</td>
</tr>
<tr>
<td>C</td>
<td>N-Noscapine</td>
<td>N-3-Methoxy Phenethyl Noscapine</td>
<td>Arrest G2 phase</td>
</tr>
<tr>
<td></td>
<td>3-Methoxy Phenethyl,3-Bromo Phenethyl</td>
<td>Phenethyl Noscapine</td>
<td></td>
</tr>
</tbody>
</table>
CONCLUSION
Natural Products plays an important and dynamic role in cancer therapeutic. Many natural compounds which are used in the treatment of cancer act by interacting with microtubule but all of them are associated with many side effects. But noscapine is one of the natural products which is free from toxic effects such as myelosuppression, peripheral neuropathy, alopecia, G.I.T toxicity, multi drug resistance which mainly occurred in other anti-cancerous natural product such as taxol, vincristine, vinblastine, etc. All these properties make noscapine a target or lead for synthesis of its analogs and other compounds for the treatment of cancer.

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