International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 2760-2772 www.ijprajournal.com ISSN: 2249-7781

The Competence of Andrographolides as Natural Armer for Treatment of Cancer
Running title: Pharmacology of andrographolides cancer treatment

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Submitted: 09-06-2023 Accepted: 19-06-2023

ABSTRACT
Andrographis paniculatais a medicinal plant traditionally used for treatment of cold and cough, fever, laryngitis, and several infectious diseases.A. paniculata extracts have demonstrated diverse effectiveness against a variety of illnesses, including cancer. The active biomolecules of A. paniculatamainly are lactone and diterpene. Andrographolide and analogues have been widely used for prevention of different diseases.Strong anti-inflammatory and anti-cancer properties have been demonstrated for andrographolides.By decreasing the proliferation of cancer cells by blocking the NF-B, PI3K/AKT, and other kinase pathways, as well as by causing apoptosis, it demonstrated potential as a chemopreventive drug. The anti-apoptotic proteins Bax, p53, and activated caspases were expressed in distinct cancer cells, and andrographolide triggered both intrinsic and extrinsic apoptosis pathways in them. In cancer chemotherapy, andrographolide was successfully employed as an anti-cancer medication. Andrographolide inhibited the growth of human breast, prostate, and hepatoma tumors. It is necessary to conduct more clinical and academic studies on andrographolide and its analogues in cancer chemoprevention. When combined with other chemotherapy medications, andrographolide has the potential to be a powerful anticancer agent.
Key words: Andrographis paniculata, lactone, andrographolide, NF-κB, anti-cancer

I. INTRODUCTION

Cancer is a condition in which cells grow abnormally and become potentially capable of invading other parts of the body leading to the group of diseases. About 18.10 million new cases and 9.6 million cancer deaths (excluding non-melanoma skin cancer), according to the GLOBOCON 2018 report on cancer, it is the second leading cause of death globally [1]. Until recently, the synthetic medications used to treat cancer had some side effects, including impaired immune systems, baldness, cardiotoxicity, female infertility, and ovarian failure etc [2-4].Neoplasms and malignant tumours are other words that are used. One defining feature of cancer is the quick development of abnormal cells that grow beyond their usual boundaries, and whicheventually move to other parts and spread to other organs; this process is known as metastasis. Widespread metastases are the primary cause of cancer patient death.

In 2020, breast cancer (2.26 million instances), lungs (2.21 million cases), colon and rectal (1.93 million cases), prostate (1.41 million cases), skin (non-melanoma) (1.20 million cases), and stomach (1.09 million cases) were the most frequently diagnosed cancers. Whereas the most common causes of cancer death in 2020 were reported for lungs (1.80 million deaths);colon and rectum (9,16,000 deaths); liver (8,30,000 deaths); stomach (7,69,000 deaths); and breast (6,85,000 deaths).The most common cancers vary between countries (Figure 1). In 23 nations, the most prevalent form of cancer is cervical. Focus should be placed on traditional medicine, which is an alternative treatment with the fewest side effects for cancer patients. For thousands of years, people have employed plants as a well-known remedy for illnesses of all kinds, particularly cancer.

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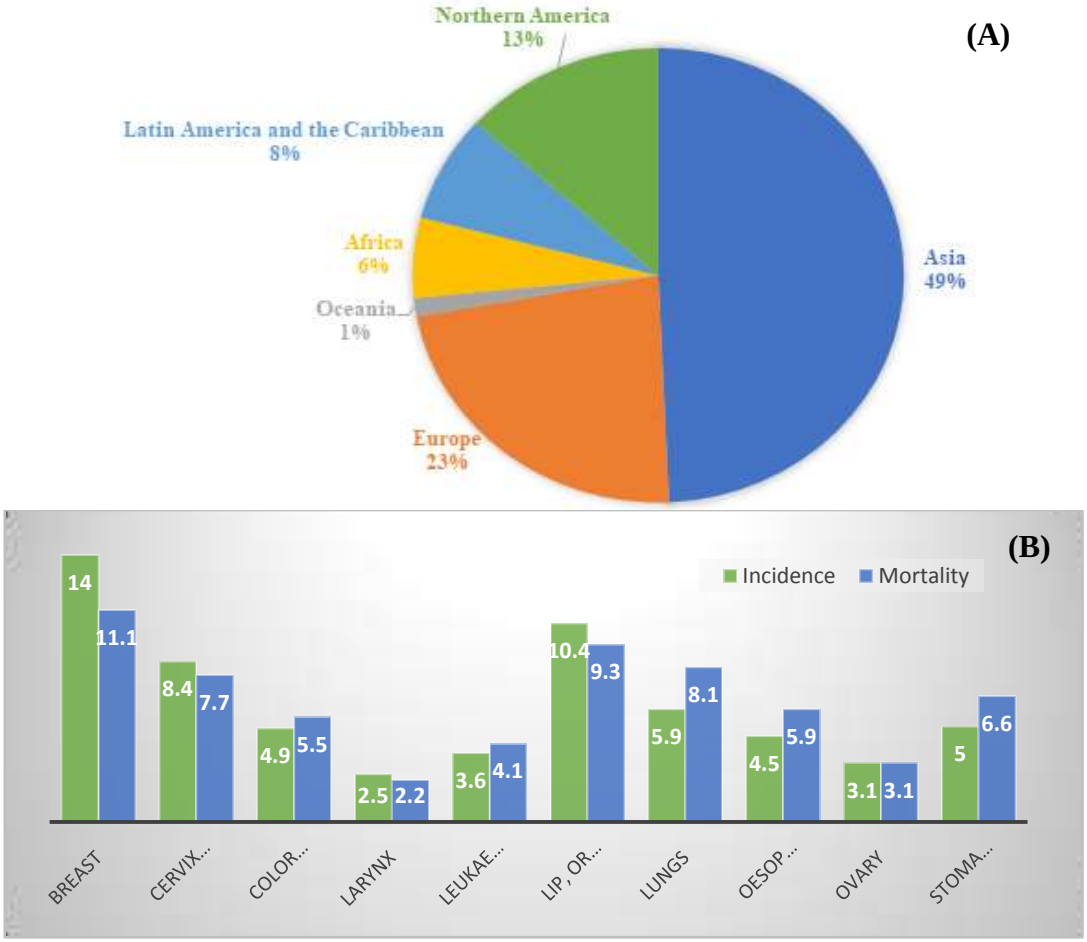

Figure 1 (A): Estimated number of new cases in 2020, all cancers, both sexes, all ages; (B) showing most common cancer cases (2018) reported in India

According to Cai et al. [5], plants provide a source of physiologically active natural products such as flavonoids, phenolics, lactones, and other chemicals that have been shown to have anticancer properties. In this review, we gathered various parameters of this plant with focus on the effects of andrographolide on cancer and the complex mechanisms involved. To elucidate the effects of andrographolide and its derivatives, we extensively reviewed and compiled literature mainly against human cancers. We elaborated on the principles of cell development and death with future implications for cancer prevention while concentrating primarily on the role of andrographolide in human cancer [5].

Ethnopharmacology of A. paniculata

Andrographis paniculata (Burm.f.) Nees, a member of the Acanthaceae family, includes several species with medicinal uses that are popular in China, India, Sri Lanka, and other South Asian nations. A. paniculata is commonly known as ‘king of bitters’ and as Kalmegh in India (Figure2). It is known as "Fah Tha Lai" in Thailand while being pronounced "Hempedubemi" in Malaysia. It is referred to as "Senshiren" in Japan and "green chiretta" in Scandinavia. It is widely used in the traditional medical systems of China, Hong Kong, Malaysia, the Philippines, Indonesia, Thailand, India, and Pakistan. A. paniculata has variety of defense response to microbes via cyanogenesis, phytohormone activation, lignifications of cell wall, alteration of secondary metabolites, and a long list of therapeutic usage in Indian and traditional medicine. Natural remedies and dietary elements have recently been connected to the prevention and risk of cancer. A. paniculata possesses a wide range of important biological

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effects, including hepatoprotective, antimicrobial, anti-inflammatory, and anti-thrombotic properties [2-3]. A. paniculata has also been used for treating animal diseases, e.g. respiratory infection and diarrhoea, as an alternative to antibiotics. Andrographolide and A. paniculata extracts have been widely employed in vitro and in vivo for a variety of pharmacological characteristics. Ethanolic extract of A. paniculata has demonstrated antiviral activity against herpes simplex virus type 1 [1,4]. This plant has been used for the treatment of neoplasm as mentioned in ancient Ayurveda.

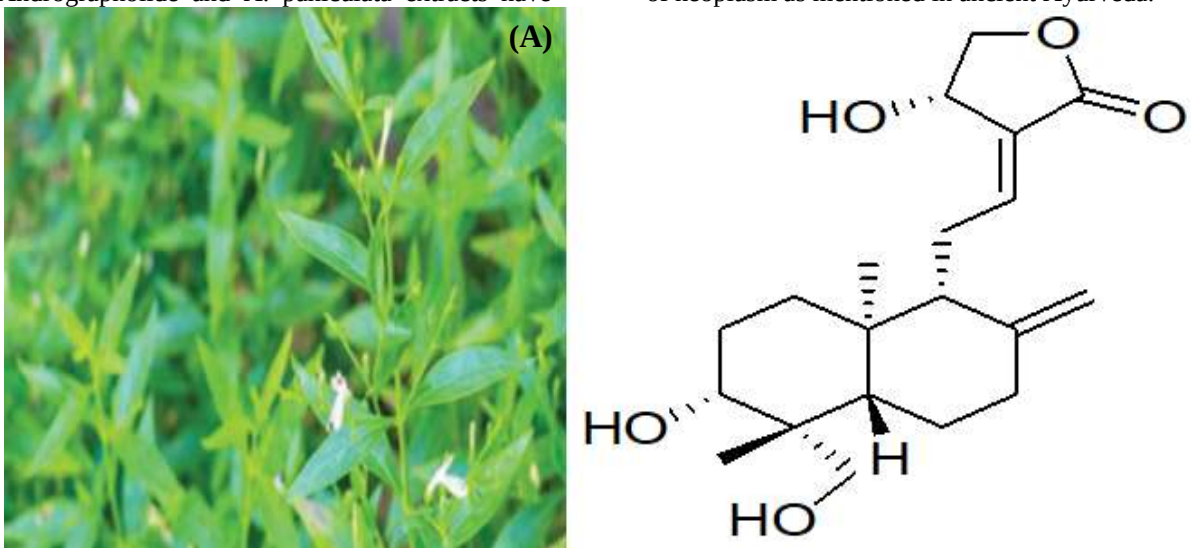

Figure 2 (A): Andrographispaniculata;(B) showing molecular structure of andrographolide

Andrographolide is the major phytochemical constituent of A. paniculata, a bitter most compound among natural products. Pharmacological studies indicate the properties of andrographolide in protection of liver and gallbladder, and have been found to be slightly more active than silymarin (a known hepatoprotective drug). Andrographolide is specifically rated very high in therapeutic action in curing liver disorders, common cough and cold, and inflammation and cancer in humans. In general, andrographolide has demonstrated effectiveness against several conditions, including allergic reactions, haemorrhagic lesions, and central nervous system malfunction.

Phytochemistry of A. paniculata

Diterpenoids, flavonoids, and polyphenols are the pre-eminent phytochemicals produced by A. paniculata. Andrographolide is the vital diterpenoid which constitute about 4.0% of entire dried plant (Fig 4), 0.80% to 1.20% of stem and 0.50% to 6.0% of leaf extract. Deoxygenated andrographolide, Neoandrograpide, Isoandrographolide, 14-deoxy-11,12-didehydroandrograhide are other underling diterpenoids. Andrographanin, andrographolide, and Neoandrographolide are isolated from roots.

Andrographolide as natural weapon against human Cancer

Andrographolide and its equivalents are mostly used to treat disorders including allergic reaction, hemorrhagic lesion, and central nervous system dysfunction. According to reports, andrographolide and its derivatives have excellent therapeutic potential against human cancer, inflammation, common colds and coughs, and liver disorders. These metabolites have been used as/in antipyretic, antiinflammatory, hepatoprotective, immunostimulant, and anti-neoplasm. The low aqueous solubility of andrographolide causes lower bioavailability subsequently used for oral administration in appropriate tissues localization therefore used in poor therapeutic purpose. Because of their short half-lives and ease of excretion through the gastrointestinal system and urine, andrographolide and its derivatives have the great property of not remaining in the body for an extended period.

It is important to recognize anticancer agents from natural sources with insignificant side effects compared to chemotherapeutic drugs. On several human cancer cell lines, andrographolides anticancer activity was assessed both in vitro and in vivo. The methanolic extract of the plant showed highest inhibition in the growth of KB (papillomavirus 18) and P388 (leukemia) cancer

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cells. andrographolide showed the maximum effect at ED50 of 1.50 µg/mL (KB cells) and 1.0 µg/mL (P388 cells) [6]. The anticancer effect of andrographolide was attributed to increased production of IL-2 (interleukin-2), TNF- (tumour necrosis factor), and CD marker expression, as demonstrated by the investigation on various cancer cell lines in a dose-dependent manner. This was also verified by the in vivo study of Rajagopal et al. [7]. The BCL-2 family's function in cancer cells was established, and it was demonstrated that AG started the process of apoptosis. Additionally, according to reports from Zhou et al. [8], andrographolide triggered caspase 8-dependent Bid cleavage, a change in Bax conformation, mitochondrial translocation, release of cytochrome c from mitochondria, and activation of caspases 9 and 3 that resulted in apoptotic cell death. When andrographolide was applied to various human cancer cell lines, it caused TRAIL-related apoptosis (extrinsic death receptor pathway; tumour necrosis factor-related apoptosis-inducing ligand) to occur. In TRAIL-resistant cells, it also caused apoptosis because TRAIL's death receptor 4 (DR4) was upregulated. Because of increased reactive oxygen species generation and c-Jun NH2-terminal kinase activation, andrographolide activated p53 by boosting its phosphorylation and protein stabilisation.Hence, it blocked the TRAIL-induced apoptosis in andrographolide prompted sensitized cells [9].

Hung et al. [10] reported that andrographolide treatment at 10 µM for 3 h sensitized Ras-transformed cells when exposed to radiation in vitro. Other studies were also conducted on cancer cell lines such as colon, prostate, breast, and leukemia.Research on colon cancer cell lines revealed that the anti-proliferative action of Lovo cells inhibited the proliferation of these cells. A drop in the formation of Cyclin D1/CDK4 and Cyclin A/CDK2 complexes inhibited the phosphorylation of Rb (tumor suppressor protein) and detachment of Rb/E2F (transcription factor) complex [11]. In another study, blocking MMP2 (matrix metalloproteinase) activity resulted in anti-invasive effect against colon cancer cell lines. Treatment with AG had no effect on the expression of MMP2 or cell adhesion regulators such -catenin and ILK (integrin-linked kinase). Extracellular signal-regulated kinase (ERK) activity with no impact on protein kinase B (Akt) activity was observed [12].

Its antitumor effect was also demonstrated in studies using prostate cancer cell lines. When andrographolide was applied to castration-resistant prostate cancer cells, its effects on AR expression resulted in a reduction in prostate cancer cell proliferation. According to Liu et al. [13], the method of blocking androgen receptor signalling has the potential to be a treatment for prostate cancer. In a study by Chun et al. [14], the effect of andrographolide on the expression of IL-6 (interleukin-6) in prostate cancer cells led to the inhibition of IL-6 expression at the mRNA and protein levels as well as the suppression of IL-6-induced cell signalling, including STAT3 and ERK phosphorylation. It reduced cell viability and triggered apoptosis in castration-resistant and androgen-stimulated human prostate cancer cells. This test did not show any significant toxicity to normal immortalized prostate epithelial cells. Additionally, studies on breast cancer cell lines were carried out. By causing a halt in the cell cycle at the G0/G1 phase, [15] tested the activity of andrographolide and its semi-synthetic counterpart, DRF 3188.

In the TD-47 human cell line, treatment with andrographolide caused DNA breakage and death in a concentration-dependent manner [16]. With a reduction in PI3 (phosphoinositide-3-kinase)/Akt activity and inhibition of OPN (osteopontin) and VEGF (vascular endothelial growth factor) expression, which are pro-angiogenic molecules, [17] reported antitumor activity against breast tumours in an orthotopic NOD/SCID mouse model. Numerous investigations using leukemic cell lines have also shown strong anticancer properties. It dramatically reduced the growth and invasion of nasopharyngeal cancer (NPC) cells. The primary processes involved in cell death and cancer inhibition were cell apoptosis, cell cycle arrest induction, and down-regulation of NF-target genes[18]. By blocking the TLR4 (Toll-like receptor 4)/NF- signalling pathway, it suppressed the mRNA and protein expression of CXCR4 (C-X-C chemokine receptor type 4) and Bcl-6 (antitumor genes) in melanoma cancer cell lines, leading to cell death [19]. When combination with other medications, it dramatically improved the growth inhibition of cancer cells due to its anti-cancer properties.

In a combined study, treatment with andrographolide and 5-FU (5-fluorouracil) caused the hepatocellular carcinoma cells (SMMC-7721) to undergo apoptosis, which was accompanied by the expression of p53, the conformation of Bax, and the activation of caspase-3, 8, and 9 enzymes. According to Yang et al. [20], adding 5-FU to

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andrographolide caused synergistic apoptosis with enhanced caspase-8, p53, and Bax activity as well as a significant change in Bax shape, which boosted cytochrome c release, mitochondrial membrane potential losses, and caspase-9 and caspase-3 activation. In contrast, bleomycin (BLM) increased anti-cancer activity in a different combinatorial study with the same cancer type by encouraging cell cycle arrest at the G0/G1 phase and upregulating the activities of capase-3 and capase-8, which ultimately resulted in cancer cells dying and tumour growth being inhibited.Andrographolide connect with numerous receptor binding sites at cell membranes and transduce certain signalling events that result in a variety of phenomena, including the induction of apoptosis, the suppression of inflammation, the arrest of the cell cycle, and the reduction of tumour growth. mitochondria; nucleus, or NC (Figure 3).

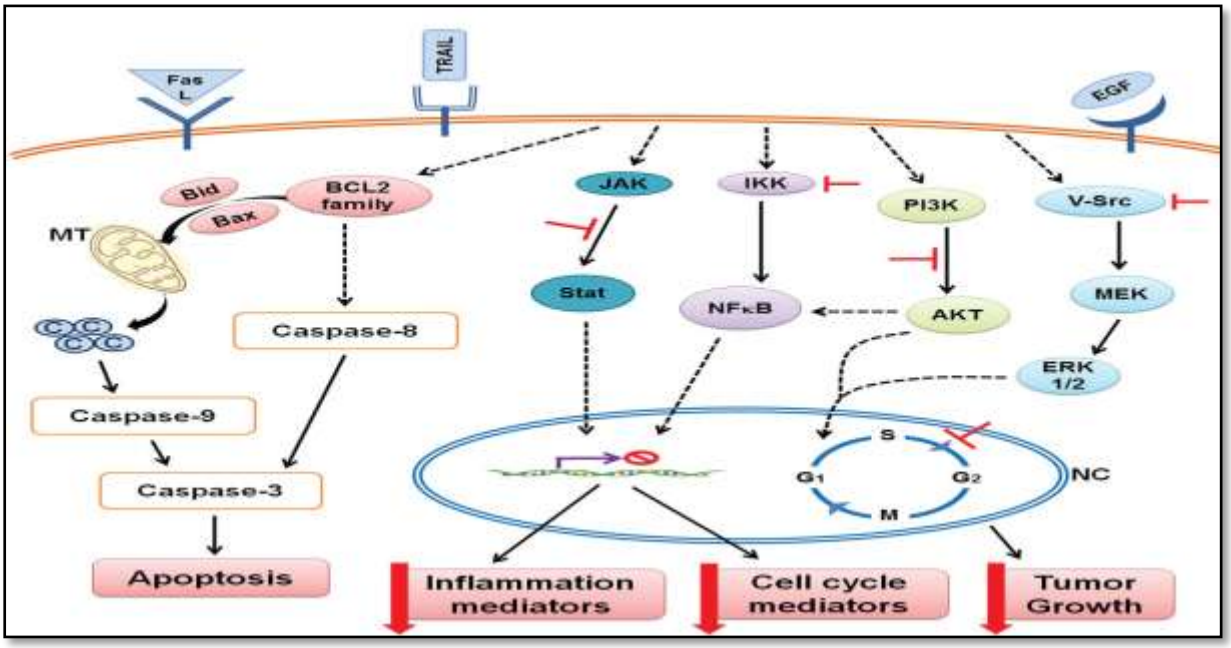

Figure 3: Schematic representation of the mechanism of action of andrographolide in cancer.

Various reports on its anti-cancer activity on lung cancer [21], oral cancer [22], pancreatic cancer [23], etc. are also reported. Both, topotecan and andrographolide showed an antiproliferative effect in U937 cells (acute myeloid) in a dose-dependent manner separately, but combination of two exhibited a synergistic effect in lower concentrations [24]. Andrographolide showed promising anti-tumor effects in in vitro and in vivo studies, established the base to initiate clinical studies for evaluating its effectiveness to prevent and/or treat cancer and its associated complications. Unfortunately, there is no report of completed trials for the anticancer activity of andrographolide. Therefore, the anti-tumor effect of andrographolide in humans is still unclear. Although, there is no clinical support that andrographolide could prevent/treat any type of cancers in humans, based on studies discussed in this review, andrographolide could play an important role as an adjuvant in the treatment of cancer.

Breast Cancer

Breast cancer is considered as a raising major life-threatening concern between the malignancies encountered worldwide in females. Traditional therapy is far from reasonable due to drug resistance and various side effects, thus a search for complementary/alternative medicines derived from natural sources with minor side effects is being emphasized. Andrographis paniculata is an oriental, traditional medicinal herb that is widely available in Asian countries. This plant's extracts show a wide range of medicinal activities, including antibacterial, antiviral, anti-malarial, and anti-carcinogenic capabilities.Andrographolide, a lactone diterpenoid, is the primary active component of this plant. This study investigates the potential mechanism of andrographolide-induced apoptosis in the highly

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proliferative and invasive MDA-MB-231 breast cancer cells, which also lack a functional p53 and oestrogen receptor (ER).

Effect of Andrographolide on Breast Cancer

Andrographolide inhibits the growth of cancer cells via several mechanisms, such ascytotoxic activity, induction of cell cycle arrest, induction of apoptosis, immunomodulatory effect, anti-inflammatory and anti-angiogenicactivities and chemoprotective mechanism. In a study conducted by Harjutaruno et al. [25], andrographolide was tested on TD-47 ERpositive breast cancer cell line.The study then showed that andrographolide induces apoptosis by increasing expression of p53 (a tumour suppressor protein), Bax protein, and caspase-3, hence exerting anticancer effects.In addition, an immunohistochemical study showed that andrographolide also reduced Bcl-2 expression. The capacity of andrographolide to stop cell cycle proliferation at the G0 and G1 phases in ER-positive breast cancer cells by severing the growth-related signalling pathway was used in another study to demonstrate the compound's anti-cancer effects. Banerjee et al.'s study, which found that andrographolide treatment on ER-positive breast cancer cells increases the production of reactive oxygen species (ROS), which contributes to the loss of matrix metalloproteinases (MMPs), activates caspase-9 and -7, and externalises phosphatidyl serine, provided additional support for this conclusion. By making these adjustments to the proteins involved in the growth signalling pathways, it is possible to stop the proliferation of ER-positive breast cancer cells.According to a 2014 study by Liang et al [26], andrographolide has antitumor characteristics that work by attenuating the Erk1/2 signalling pathway and targeting the level of the oncoprotein v-Src. Andrographolide inhibits the expression of CDK-4, which indirectly results in cell cycle arrest in the G1 phase, through regulating the expression of cyclin-dependent kinases (CDKs). Additionally, andrographolide inhibits p27-induced cell growth in ER-positive breast cancer cells. Additionally, andrographolide prevents 12-O-(TPA) produced by the invasion of ER-positive breast cancer cells. The upregulation of heme oxygenase and the downregulation of MMP9 expression may have contributed to this suppression. NF-B, which serves as the primary switch in the proliferation of ER-positive breast cancer cells, is finally suppressed as a result of the inhibition of most of the growth signalling pathways mentioned above. Therefore, by 'turning off' this primary switch, the proliferation of ER-positive breast cancer cells can be suppressed, avoiding the emergence of breast carcinoma. Andrographolide decreased cell proliferation, invasion, migration, and cell cycle arrest in a different study utilising gastric cells while promoting apoptosis in SGC7901 cells.

Colorectal Cancer (CRC)

Colorectal cancer (CRC) is one of the highest occurring malignancies worldwide. In 2020, approximately 147,950 individuals were diagnosed with CRC with a 35% mortality rate. Patients under the age of 50 have a death rate of about 20% [27-29]. Targeted therapy, chemotherapy, and surgery are examples of traditional treatments. The current chemotherapeutic drugs treat CRC, but that cause adverse toxicity. It also develops drug resistance.Although the targeted therapy is highly promising, it can occasionally be ineffective, expensive, and associated with negative side effects [30-31]. Currently, phytochemicals are viewed as a natural defence against the danger of cancer. The main benefit of using phytochemicals is that they can treat cancer with minimal or no side effects [32-33] (Table 1).

Table 1: Effect of andrographolide on colorectal cell lines (researches have been done so far)

Author	CRC cell lines	Effects
[28]	CRC LoVo	Increased cytotoxicity, apoptosis, and Bax protein
[34]	CCD841 and HT29	Increased antioxidant property Inhibition of CRC development genes
[35]	T84, HCT116 and COLO 205	Increased ER stress and apoptosis
[29]	HCT116/5-FUR cells	Up-regulation of Bax protein
[36]	HCT-116	Down-regulation of c-MET pathway Stimulation of 5-FU- induced anti-tumor effect

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[37]	SW620	Inhibition of TLR4, NF-kB-p65, and MMP-9 signaling pathways
[38]	HT29	Stimulation of Cell cycle arrest, apoptotic cell death, PARP1, p53
[39]	HCT-116	Down-regulation of PI3K-AKT-mTOR signaling pathway

Hepatic Cancer

The Hepatitis C virus (HCV) is an enveloped virus that is a member of the Hepacivirus genus of the Flaviviridae family. Its 9.6 kb genome codes for a single polyprotein that is split up into 10 pieces by viral and cellular proteases. These pieces include four structural proteins (C, E1, E2, and p7) and six non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) [40].

HCV is the main cause of liver fibrosis, cirrhosis, portal hypertension, hepatic failure, and hepatocellular carcinoma (HCC); [41] estimates that more than 170 million people worldwide have HCV infection. There is no available vaccine to prevent HCV infection. The current HCV therapy, a combination of pegylated IFN-α (PEG-IFN-α) plus ribavirin, is effective in only approximately 50% of cases and provokes severe side effects, including depression, fatigue, flu-like symptoms, and haemolytic anaemia [42-43]. Currently, telaprevir (Incivek; Vertex Pharmaceuticals Incorporated, Cambridge, MA, USA) and boceprevir (Victrelis; Merck Corporate, Whitehouse Station, NJ, USA), two NS3/4A protease inhibitors approved by the US Food and Drug Administration (FDA), show higher sustained virological response rates for the treatment of genotype 1 chronic hepatitis C [44]. Drug-resistant genotypes and mental reactions to both inhibitors, however, have been seen in clinical investigations [45-46]. This may limit the efficacy and utility of the existing HCV triple treatments and impede the development of IFN-free therapy. Therefore, it is necessary to create alternative anti-HCV drugs that are more effective and have better side-effect profiles for anti-HCV treatments. In this study, we screened many natural compounds using an HCV subgenomic replicon system in order to find one powerful agent, andrographolide, that has anti-HCV activity.

Effect of Andrographolide paniculata on Hepatic Cancer

In HCV replication and HCVcc infectious systems, andrographolide reduced HCV replication in a time- and dose-dependent manner. Andrographolide has shown a significant synergistic interaction with HCV NS3/4A protease or NS5B polymerase inhibitors IFN-, telaprevir, or PSI-7977. The enzyme haeme oxygenase-1 (HO-1) was upregulated by andrographolide, which raised the amount of the enzyme's metabolite, biliverdin. Biliverdin was discovered to inhibit NS3/4A protease activity and prevent HCV reproduction. The fact that a HO-1-specific inhibitor or HO-1 gene knockdown significantly reduced these antiviral effects suggests that HO-1 played a role in andrographolide's anti-HCV activities. It was discovered that andrographolide's anti-HCV effect was connected to its activation of p38 MAPK phosphorylation, which in turn increased HO-1 production via nuclear factor erythroid 2-related factor 2 (Nrf2).HO-1 up-regulation has been seen in various viruses, including HIV [47] and spring viraemia of the carp virus [48], as a host defensive mechanism against viral reproduction. Amplification of HO-1 has been linked to cytoprotection in chronic hepatic inflammation caused by its reaction products in addition to its antiviral effects [49]. According to Buhler and Bartenschlager[50], chronic inflammation brought on by HCV infection may be a risk factor for the onset of cirrhosis and HCC. Therefore, additional research into andrographolide's anti-inflammatory properties is desirable. According to recent research Chen et al. [51], andrographolide administration may be helpful in treatment approaches for HCV-associated liver illnesses since it can cause autophagic cell death in human liver cancer cells at high concentrations.

- Andrographolide increases the formation of the HO-1 product biliverdin, which activates the IFN response against the virus and inhibits the action of the HCV NS3/4A protease.
- In HCV replicon cells, andrographolide increases HO-1 mRNA and protein levels.
- Anti-HCV activity of andrographolide relates to an Nrf2-mediated rise in HO-1.
- IFN- or viral target inhibitors work synergistically with andrographolide to limit HCV replication.

Lungs Cancer

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Lung cancer is the main cause of the cancer related deaths globally. More than 80% of all occurrences of lung cancer are caused by non-small cell lung cancer (NSCLC). Many patients with early-stage NSCLC may not have significant clinical symptoms, which could prevent them from having surgery. A crucial enzyme in the tricarboxylic acid cycle called Na^+-K^+-ATPase is a heterodimer made up of one α-subunit and one β-subunit. The α-subunit is a transmembrane protein, which mediates the exchange of intracellular Na+ and extracellular K^+ and thus plays a key role in maintaining dynamic balance of the ions on both sides of membrane.The transmembrane potential of the mitochondria may change as a result of a variety of circumstances that influence Na+-K+-ATPase activity, causing the release of substances linked to apoptosis. Na+-K+-ATPase is said to be important for mediating cell adhesion and signal pathway activation, which is related to the incidence of lung cancer.

There are strong links between the Na+-K+-ATPase activities and the development of cancer incidence, which leads to the conclusion that the Na+-K+-ATPase may be a major target of the anti-cancer drugs for NSCLC [52]. Additionally, the transforming growth factor-1 (TGF-1) and vascular endothelial growth factor (VEGF) play a role in the adherence of cancer cells to encourage tumour spread.The innovative molecular therapy may aim to block a particular molecular pathway involved in the growth and invasion of the tumour. Because it can be employed alone or in conjunction with other treatments to boost the sensitivity of radiation and chemotherapy for NSCLC, the development of an anti-cancer therapeutic agent with minimal toxicity is crucial.

Effect of Andrographolide on Lungs Cancer

Andrographolide decreases the viability of H3255 cells and increases the quantity of LDH secreted by the H3255 cells. Andrographolide decreases levels of VEGF and TGF-β1 [53]. ELISA was used to assess the amounts of VEGF and TGF-1 expression in the cells. Inducing cancer cells to undergo apoptosis, which is characterised by DNA fragmentation, is one of the fundamental goals of tumour treatment. This study showed that andrographolide reduces the proliferation of H3255 cells, probably by increasing DNA fragmentation, lowering Na^+-K^+-ATPase activity, and reducing VEGF and TGF- production. The effects of andrographolide included a decrease in cell viability and an increase in DNA fragmentation.

According to one theory, andrographolide enters cells and causes cell apoptosis. Na^+-K^+-ATPase activity in lung cancer cells was lowered by Andrographolide, indicating dysfunction of the -subunit and/or damage of the mitochondrial membrane. This finding also raises the possibility that the mitochondrial dysfunction brought on by Andrographolide may cause lung cancer cells to undergo apoptosis. VEGF and TGF-β1 are both angiogenic factors. TGF-β1 can stimulate metastasis of Non-Small Cell Lung Cancer (NSCLC) [54-55]. It is therefore inferred that low expression of VEGF and TGF-β1 may indicate the inhibitory effect of andrographolide on angiogenesis and metastasis of lung cancer cells.The mechanisms in tumour cells that facilitate the growth and invasion of tumours are connected to PCK activation. PCK activity and overexpression can promote NSCLC metastasis. This study has demonstrated that andrographolide therapy efficiently prevents PCK activation in lung cancer cells, suggesting that andrographolide may potentially suppress the activity of tumor-related pathways and hence slow the growth of lung cancer [56-57].

Clinical trials of Andrographolide

The goal of theoretical research is to apply safe and effective drugs to clinical practice successfully. Thus, it is extremely essential to carry out large-scale randomized controlled trials scientifically and reasonably to confirm whether andrographolide is effective in the treatment of related disorders in clinical practice. We thoroughly examined ClinicalTrails.gov's website using "Andrographolide" as the keyword (https://clinicaltrials.gov/) and found that there are some ongoing projects related to the clinical researches of andrographolide (at June 24, 2021), which these diseases mostly involved in primary progressive multiple sclerosis, colorectal cancer, acute tonsillitis, acute bronchitis, esophageal squamous cell carcinoma, migraine and so on [58](Table 2). These clinical studies have increased andrographolide's credibility in the prevention and treatment of associated disorders, which will hasten the clinical development of its products.

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Table 2: Clinical trials for andrographolide in the prevention and treatment of related diseases registered at ClinicalTrials.gov/

Study title	Conditions	Status	Identifier
Efficacy, safety, and tolerability of andrographolides versus placebo in patients with progressive forms of MS	Primary progressive multiple sclerosis, multiple sclerosis, secondary progressive	Unknown, phase 1/2	NCT02273635
Study of andrographolides with or without capecitabine to treat colorectal cancer	Colorectal neoplasms	Terminated, phase 2	NCT01993472
Evaluate the efficacy and safety of andrographolide sulfonate in patients with acute tonsillitis	Acute tonsillitis	Unknown, phase 4	NCT03134443
Evaluate the efficacy and safety of andrographolide sulfonate in patient with acute bronchitis	Acute bronchitis	Unknown, phase 4	NCT03132623
Magnesium, vitamin B2, feverfew, Andrographis paniculata and coenzyme Q10 for episodic migraine prophylaxis	Migraine	Completed, N/A	NCT04463875
The effect of Andrographis paniculata (AP) on palliative management of advanced esophageal cancer	Squamous cell carcinoma of esophagus	Recruiting, phase 3	NCT04196075
Efficacy study of Andrographis paniculata purified standardized extract (ApE) in patients with multiple sclerosis (MS)(PCNS-EM)	Multiple sclerosis, relapsing–remitting	Completed, phase 1/2	NCT02280876
Pharmacometabolomics of Andrographis paniculata and metformin in healthy volunteers under fasting condition	Pharmacokinetics molecular mechanisms of pharmacological action	Recruiting, phase 1	NCT04161404
A study of andrographolide sulfonate in patients with acute exacerbation of chronic bronchitis	Acute exacerbation of chronic bronchitis	Unknown, phase 4	NCT03132610

Future Prospects

Natural products have shown significant contributions in anticancer therapies. Several potent and effective anticancer agents, such as aspirin, vincristine, vinblastine and paclitaxel, are derivatives of plant-derived bioactive molecules. In numerous nations, including India, Andrographis paniculata has been utilised for medicinal purposes in traditional medicine. Andrographolide is a key bioactive compound with anti-inflammatory, antiviral, analgesic, immunosuppressive, and antipyretic effects. The cumulative effects and mechanism of action of andrographolide has been represented in Figure 3. Andrographolide and analogues induced apoptosis in various cancer cells and caused cell cycle arrest, and showed antitumor properties.In both animal and human cancer cells, andrographolide and its equivalents caused cell cycle arrest and death, obstructed metastasis, and decreased angiogenesis. The main modes of action

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of andrographolides involved downregulation of mediators of cell cycle progression, inflammation, metastasis, and angiogenesis, as well as inhibition of v-Src, NF-B, STAT3, and PI3K/AKT activity.

II. CONCLUSION

For the development of anticancer drugs, andrographolide and its analogues have been the focus of intensive chemico-biological research. In both in vitro and in vivo settings, several andrographolide analogues have demonstrated superior anticancer activity. A few malignancies, including breast, cervical, lung, colon, liver, and prostate carcinomas, were affected by andrographolides.However, the principal drawbacks that hinder its clinical use in cancer chemoprevention are its poor oral bioavailability, high lipophilicity, low aqueous solubility, bitter taste, strong affinity to proteins, and brief half-life. It is reported that the combined anticancer and immunomodulatory activities of andrographolides can be effective without any immunological side effects. Andrographolides can be a very effective medicine for the treatment of any type of cancer, either by itself or in conjunction with other medications. Numerous investigations on its derivatives revealed outstanding anticancer properties in both in vitro and in vivo settings. Although some studies discussed the pharmacokinetics and therapeutic effectiveness of andrographolides in human clinical trial models, nothing were reported in cancer treatment. In many combination therapies for cancer, andrographolides is used as an adjuvant, either to increase the efficacy of the chemotherapeutic medication or to address the issue of chemotherapeutic drug resistance. To confirm the pharmacological, pharmaceutical, and toxicological effects of andrographolide, additional clinical and biological studies are required. In addition, combined drug discovery and combinatorial studies with andrographolide analogues may serve helpful in cancer therapeutics.

III. ACKNOWLEDGEMENT

The author feels privileged to thanks Lingaya's Vidyapeeth, Faridabad, Haryana for providing all sorts of support. The Authors are also thankful to Dr Varisha Anjum, SUSU, Russia for all the support and guidance in writing a paper.

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