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Cucurbitacins and Cancer: A Review on in Vitro, in Vivo, in Silico Analysis of Compounds against Estrogen Receptor (ER) for a Better Treatment

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Abstract:- New diseases and germs are emerging day by day in our world. But cancer is the most deadly disease that came many years. It is very sad that it could not be eradicated permanently till today. Cancer is caused by abnormal cell growth. As a result it is impossible to save the victim. So it is a life-killing disease. But many scientists are still struggling to find a cure for it. And many types of plants, fruits, vegetables are being researched to use the chemicals against cancer. In this study, explain the anticancer properties of a plant family known as Cucurbitacins. Estrogen signaling is crucial for carcinoma initiation and progression which stimulates estrogen receptor (ER) for proliferation leading to growth of estrogen-responsive tumors. Plant compounds like coumarins, cucurbitacins, flavonoids, alkaloids and flavonoids, etc...From different plant parts were used because the further alternative treatment of breast cancer. Among the compounds, cucurbitacins have shown promising results over the treatment. Cucurbitacins is a highly oxygenated tetracyclic triterpenoids species. These include the blockers of the JAK/STAT pathways. Now explain the chemical structure, physical properties, and the role of in vitro, in vivo, along with the in silico analysis shows that the estrogen receptor controls the proliferation of breast cancer cells.

Keywords:- Cancer, Estrogen receptor, Cucurbitacins, JAK/STAT, MAPK and Proliferation.

I. INTRODUCTION

Cucurbitacins are one of the major classes of a highly oxygenated tetracyclic triterpenoids. They may be notably dispensed within the plant nation where they act as heterologous chemical pheromones that shield plants from external biological insults. Chen X said "The value of their broadspectrum pharmacological bioactivities was first attracted interest in the 1960s" (*Chen et al.,2012)*Lang to said "natural and semi-artificial cucurbitacins show promising anticancer activities starting from anti-proliferation, mobile cycle arrest to induction of apoptosis"(Lang *etal*.,2012,Pan *et al.*,2012). Rios said "Till, the prevailing date more than 40 new cucurbitacins and cucurbitacin-derived compounds have been remoted from the cucurbitaceae circle of relatives and from unique species of the plant nation" (Rios *et al*.,2012, Devita*et al.*,2011). The massive of cucurbitacins are considered to be selective inhibitors of the JAK/STAT pathways; but, other mechanisms can be implicated in their apoptotic results, which includes to Rios and Devita said "the MAPK pathway (recognised to be crucial for most cancers cell proliferation and survival), PARP cleavage, expression of lively caspase-three, decreased pSTAT3 and JAK3 ranges, similarly to decreases in severa downstream STAT3 desires together with Mcl-1, Bcl-2, Bcl-xL and cyclin D3, all of which are implicated in apoptosis and the mobile cycle manipulate"(Rios *et al*.,2012, Devita *et al*.,2011). The structural composition of the following cucurbitacins are recognised and were specified with The aid of the letters:Q, B, C, E, F, G, D,H, I, J, K, L, A,O, P, R and S. Sun said "Cucurbitacin I triggered reduction of boom in breast and prostate carcinoma cell strains (MDA-MB-231, MDA-MB-468, Panc-1), *in vitro*, in addition to in nude mice xenograft models" (Sun *et al*.,2008). Cucurbitacin Q induces apoptosis greater potently in human and murine tumors. "Furthermore, in HeLa cells, cucurbitacins inhibited DNA, RNA, and protein synthesis" (Duncan *et al*., 1996). Duncan said "Cucurbitacin E inhibits the proliferation of prostate most cancers cells and taken approximately disruption of the cytoskeleton shape of actin and vimentin"(Duncan *et al*., 1996). They were said to "own a wide range of organic effects which includes chemoprevention and hepatoprotection, as well as anti-inflammatory, antimicrobial and antitumor activities."

II. CHEMICALS STRUCTURE OF CUCURBITACINS

According to the 2008 review of Sun J, cucurbitacins are structurally identified by their tetracyclic cucurbitane nucleus skeleton (triterpenes). Triterpenes are made up of six isoprene devices in their basic structure. Therefore, triterpenes are C30-compounds. The hypothetical triterpene hydrocarbon cucurbitane, also known as 19-(109-)-abeo-five alpha-lanostane (also known as nine-methyl-19-nor-lanosta-5-ene), is the source of cucurbitacins, which are divided into twelve categories according to the characteristics of their structural features (Sun *et al*., 2008). Cucurbitacins I and D are acetylated to produce cucurbitacins E and B, which increases their hydrophobicity and cytotoxicity (Chen *et al*., 2005).Cucurbitacins E and I differ from cucurbitacins B and D, respectively, by the existence of a double bond between C1 and C2, which enables you to increase both the hydrophobicity and the cellular toxicity, according to a 2005 statement by Chen; (Chen *et al*.,2005).

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III. PHYSICAL PROPERTIES

According to Duncan, cucurbitacins are typically crystalline substances at room temperature. The cucurbitacins' chemical makeup reveals that they have hydrophobic structures, which results in a negative water solubility. Duncan and others (1996) Only a small number of polymeric micellar structures have so far demonstrated favourable benefits in the tumor-focused delivery of poorly soluble capsules following systemic administration. (Liu *et al*., 2008; Duncan *et al*., 1997) Nanoscale carriers (20–100 nm in size) having a hydrophilic shell and hydrophobic centre, polyethylene oxide block micelles have shown exceptional promise in the solubility and controlled transport of hydrophobic medicines. Duncan and others (2007) Micelles of cucurbitacins I and B made of poly (ethylene oxide)-block-poly (caprolactone) (PEO-b-PCL) and poly (ethylene oxide)-block-poly (benzyl carboxylate)-caprolactone (PEO-b-percent) (determine 1. G, B), which three are signal transducer and transcription activators inhibitors (STAT3). It was determined that the polymeric micellarcucurbitacins' anti-cancer and STAT3 inhibitory effects on a B16-F10 cancer cell line in vitro were comparable to those of free capsules. Cucurbitacin I (parent 1.G), injected intravenously, caused established B16-F10 mouse melanoma tumours to regress in vivo. PEO-b-percentmicellarcucurbitacin I was found to provide comparable anti-cancer results in opposition to B16- F10 tumours and to limit drug levels in animal serum while maintaining high drug stages in tumour after intra-tumoral treatment, in contrast to free cucurbitacin I (Figure 1.G).

IV. CUCURBITACIN AND ANTITUMOR ACTIVITY

Very few information exist regarding the positioning of cucurbitacins at the molecular level, which has caused cucurbitacins' slow but steady growth as anticancer agents. (2007) (Kee*etal*.). Growth suppression, mobile cycle arrest in the G2/M segment, and induction of apoptosis in cancer cells are all objectives of cucurbitacin motions. In 2000, Liu *et al*. The Janus kinase/sign Transducer Activator of Transcription 3 (JAK/STAT3) signalling pathway, which has to be activated for cell proliferation and maintenance, is one of the mechanisms underpinning the anti-tumorigenic potentials of cucurbitacins. (Bowman and colleagues, 2000) Cucurbitacin I has been shown to decrease phosphotyrosine STAT3 in cancer cell lines and human malignant lung cells. (2003) Blaskovich*et al*. According to several reports, cucurbitacin E reduced tumour angiogenesis by preventing the signalling pathways for JAK-STAT3 and mitogen-activated protein kinases (MAPK) (Dong *et al*., 2010). Cucurbitacin B and E's anti-proliferative properties have been linked to the location of actin cytoskeleton interference. The disruption of the F-actin cytoskeleton has been linked simultaneously with the anti-proliferative sports. (2000) Duncan *et al*. It has been suggested that the combination of Cucurbitacin B and docetaxel may also enhance the therapeutic benefits of chemotherapy by suppressing STAT3 in laryngeal cancer patients. (2000) Liu *et al*. Given that cucumber results have been declared to contain cucurbitacin C, it is anticipated that they would have anti-tumor effects. (2002) Higashio*et al.*

- **IN VITRO:** chemicals and cells. The following cell lines were kept alive: MCF-7, MDA-MB-231, MDA-MB-453, T47D, BT474, SK-BR-3, and ZR-75-1 cells (American kind tradition collection, Manassas, VA, USA) in Roswell Park Memorial Institute media (RPMI)-1640 (Invitrogen, Carlsbad, CA, USA) with 10% foetal bovine serum (FBS; Invitrogen), 10 U/mL (Invitrogen). CuB was graciously provided by CK Life Sciences International Inc., Hong Kong.

  - **RESULT:** Various human breast cancer cell lines are interested in CuB because it possesses antiproliferative properties. Using cell counts at 40.8, the antiproliferative activity of CuB on a broad array of human breast cancer cells lines was assessed. All breast cancer cells strains saw their growth effectively reduced by CuB. Although the SK-BR-three cells' growth curve is not depicted, their expansion was likewise muted. The MDA-MB-231 cells were most sensitive to CuB (ED50, 3.03 108 M, table 1), and the mean effective dosage that inhibited 50% growth (ED50) ranged from 3.03 108 to 4.18 107 M.No correlation was found between the cells' sensitivity to CuB and their various capacities, such as ER famous, HER-2/neu amplification, or p53 mutation (table 1).

Effect of cucurbitacin B (CuB) dose-dependently on 6 human breast cancer cell lines. At a density of 4 103 cells/well, cells were grown on 12-well plates for 48 hours with either zero. CuB at various quantities or 1% dimethyl sulfoxide (DMSO) (diluent management).Hemocytometers were used to determine cell counts. Findings are presented as a percentage of control, with results for untreated control cells set at 100%. The results are the mean SD of three studies performed on samples in duplicate.

Relative sensitivity	, ER	, HER-2/neu	P53 mutation	cell line	ED50 (M)
0.07	-		+	MDA-MB-231	3.03 × 10−8
0.08	+	amplified	-	ZR-75-1	3.20 10-8
0.09	+		-	MCF-7	3.63 × 10−8
0.28	+		+	T47D	1.18 10-7
0.33	+	amplified	+	BT474	1.36 107
1	+	amplified	+	MDA-MB-453	4.18 x 10-7

Table 1: Effect of cucurbitacin B on proliferation of breast cancer cell lines

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- **IN VIVO:** experiments on animals. MDA-MB-231 breast cancer cells (1 106 in total) were resuspended in 100 l of matrigel and isotopically injected into the mammary fat pads of lady nude mice. The mice started receiving intraperitoneal injections of one mg/kg of CuB three times per week on the day following cell inoculation. Every week, measurements of the peak, transverse and longitudinal diameters were taken. The extent become calculated through multiplying these factors; and the relative tumour size become determined by way of dividing the product via the initial extent. After 6 weeks, the mice were sacrificed to weigh the dissected tumors. At that point, blood turned into taken for analysis; organs and tumours have been inspected, dissected, constant and stained with hematoxylin–eosin and/or Ki-67.

  - **RESULT:** CuB prevents mice kept naked from developing MDA-MB-231 tumours. We tested CuB's anticancer activity against MDA-MB-231 human breast cancer cells orthotopically generated into the breasts of woman nude mice in order to ascertain its capacity to prevent tumour growth in vivo. Treatment started the day after implantation and consisted of either a car control or intraperitoneal injections of 1 mg/kg/day of CuB three times per week. By using calliper measurements, tumour volumes have been tracked every week. CuB significantly reduced the growth of MDA-MB-231 tumours (by 50.1% vs manipulate) (Fig. 1). The CuB-treated mice looked and behaved like car-handled mice, save from the fluid in their stomachs.Mice were slaughtered after 6 weeks of CuB treatment to weigh the dissected tumours, assess blood cell counts and chemistries, and perform gross and microscopic examinations of the tumours and other organs. Table 2. The excised tumours in the CuB-treated mice weighed noticeably less than those in the car-treated mice (0.87 g versus 1.29 g for CuB versus vehicle) (Fig. 2). CuB-treated mice exhibited chylous ascites in their abdomens, and a microscopic examination revealed that it was full of lymphocytes. White blood cell levels in the peripheral blood were significantly decreased when compared to the untreated group (P = 0.03).Cucurbitacin B (CuB) exerts greater inhibitory effects on orthotopically positioned human breast cancer cells in naked mice. Animals were divided into two groups at random the day after tumour injection and given I.P. treatments of either car (n = 9) or 1 mg/kg/day of CuB (n = 9). Using a pupil's t-test for statistical analysis, car-manipulated animals and CuB-treated animals were compared.

	P-value	untreated	CuB
Count of peripheral blood cells			
White blood cells (103/L):	0.03	6.4 ± 2.2	3.5 ± 2.5
Lymphocytes (103/L)	0.04	4.4 ± 1.6	1.6 ± 2.0
Red blood cells	0.57	9.2 ± 0.5	9.4 ± 0.6
Hemoglobin (g/dL):	0.33	14.0 ± 0.5	13.8 ± 0.3
Platelet count per litre (103/L)	0.03	280.0 ± 141.2	494.3 ± 117.7
Total protein in the serum (g/dL)	0.34	4.6 ± 1.0	4.4 ± 1.0

Table 2: Change of peripheral blood cell count and serum chemistry

A comparison of the weights of surgically removed human breast cancer tumours from untreated and CuB-treated mice. Mice were put to death six weeks after receiving I.P. treatment with either vehicle or CuB 1 mg/kg/day, and tumours had been examined and measured. The effects of nine experimental and nine managed malignancies comprise the suggest and SD.

- **IN SILICO*:*** statistical assessment. The manner SD has been used to present the facts. When analysing sets of data, students use a t-check (two-tailed) to determine the statistical significance of their findings. The asterisks in the figures indicate significant variations between the experimental groups and the corresponding manipulate situation. P-values less than.05 were considered statistically significant.

V. CUCURBITACIN B - ORGANIC DELIVER CHAIN AND FUTURE SCOPE:

The botanical origin and artificial manufacture of cucurbitacin B have been extensively investigated in recent years. Jung and Lui (Jung et al.,2010) proposed convergent edifice efforts. According to Razavilar and Choi (Razavilar et al.,2014), the diffusivity of cucurbitacin B is dependent on the wiggling motion of the block copolymers to diffuse concurrently with the contemporaneous water molecules diffusion via a hopping mechanism. Toker et al. (Toker et al., 2003) developed a novel method for increasing cucurbitacin B production by the use of Ecballium elaterium callus culture. They demonstrated that cultured calluses of stem explants incubated in media supplied with 1 mg/l benzyl adenine and 0.1 mg/l naphthalene acetic acid could appropriately accumulate the chemical.Mei et al. (Mei et al.,2016) proposed a method for cucurbitacin B bioproduction from one of its figure glycosides using a particular Streptomyces sp., incubation in enzyme broth, and extraction with ethyl acetate. The approach became modest, simple, and prolific; yet, its hydrophobic and electrostatic structure made it prognostically challenging. Rapid administration, accompanied by diminished performance of

cucurbitacin B, may also alleviate serious uncertainties about the treatment mode and dose in practical application. Cucurbitacin B is hydrophobic and so has poor in-situ absorption. Its absorption from carboxymethyl chitosan films using phospholipid-bile salt-combined micelles as mucoadhesivebuccal films resulted in a two.69-fold increase in bioavailability (Lv et al.,2015), Molavi *et al* (Patol *et al*.,2009) organized polymeric micelles of much less than ninety nanometers by means of encapsulating cucurbitacin B in PEO-b-PCL and PEO-b-p.c.. PEO-b-PCL micelles confirmed superiority in maintaining a sustained release of hydrophobic cucurbitacin B, thereby proscribing the rate of STAT3 activation in murine cancer cell line. Molavi et al (Patol et al.,2009) created polymeric micelles of fewer than 90 nanometers by encapsulating cucurbitacin B in PEO-b-PCL and PEO-b-p.c. PEO-b-PCL micelles shown advantage in sustaining the release of hydrophobic cucurbitacin B, limiting the pace of STAT3 activation in a murine cancer cell line. Using molecular dynamics simulation studies, Patel et al (Chang et al., 2015) predicted that raising the PCL/PEO ratio in PEO-b-PCL-cucurbitacin B micelles would reduce the drug release charge due to polar intermolecular interactions. Docking strength investigation indicated that an increase in ratio may need the establishment of additional hydrogen bonds between the molecule and poly (-caprolactone).In 2015, Cheng et al (Wang et al., 2010) created a system of berberine hydrochloride altered phospholipid complex loaded cucurbitacin B (CUB-percent-BER) and investigated its transport and effectiveness against cholangiocarcinoma. Berberine hydrochloride is supposed to increase bile release and so contribute to sustained and extended drug release. Cucurbitacin B with phospholipids and berberine hydrochloride had a higher medicine shipping cost and was more cytotoxic to most cancer cells in vitro and in vivo. Cucurbitacin B was encapsulated in glycosylated solid lipid nanoparticles by Wang et al (You et al., 2015). The system's management resulted in a large increase in focused cytotoxicity, with an average aim specific performance of about sixty four%, whereas traditional formulations confirmed best 23-26%.You and colleagues (Kausar et al.,2013) predicted a direct relationship between STAT3 protein localisation and autophagy. STAT3 in the cytoplasm suppressed autophagy via EIF2AK2 sequestration and interaction with FOXO1/3. Cytoplasmic location caused direct protein-to-protein interactions and hampered autophagy by interacting with autophagy-related proteins. Nuclear STAT3 regulated autophagy by transcriptional control of autophagy-related genes, including the BCL2 family. It is mediated at the genetic level, with STAT3 activation also causing genomic remodelling and autophagy attenuation.Mitochondrial translocation of STAT3 constitutively suppressed autophagy. According to studies, cucurbitacin B and its derivatives inhibited STAT3 activation (Zhang M et al.,2014 ,Zang YT et al.,2014, Chen et al.,2010,Liu et al.,2008 , Liu et al.,2008, Zhang et al.,2012, Morostica et al.,2015, Liu et al.,2010, EI-senduny et al 2016,Liu et al.,2008,Yar saglam et al.,2017–Kim et al.,2017, Yang et al.,2017,Duangmano et al.,2012 ,Seo et al.,2014). Autophagy signalling is a relatively recent area of study. Cucurbitacin B and its involvement in autophagy are mostly unknown, leaving a large gap to be filled.

VI. CONCLUSION

Despite the fact that Cucurbitacins are relatively dangerous substances, and their biological sports are frequently close to their deadly dosage level, those compounds have enormous pharmaceutical potential. Aside from their toxic character, cucurbitacins have been shown to have therapeutic efficiency against irritation, major malignancies, arteriosclerosis, and diabetes. The opinions on their toxicity must not eclipse their ability to be used as great medical agents. The chemical modification of several useful groups of these compounds to reduce harmful results may also provide essential lead compounds for future research. Numerous Cucurbitacin analogues have been investigated and are well positioned for poisonous nature and efficacy against tumour cell lines.The role of Cucurbitaceae species in empirical diabetes control has been highlighted in modern medication development from medicinal plant life. It's fascinating to see that most of the traditionally used natural flora for diabetes, primarily from the genus Momordica, are high in triterpenoids, Cucurbitacins, and related chemicals momordicosides. It is considered that their frequency is greater in the roots and end result of such plants. Data on the absorption, distribution, metabolism, and excretion of these substances is limited, and this may be an area of investigation to preserve in question their harmful effects in animals. Unattended medicinal leads from nature research may also prove to be of significant relevance in generating scientifically proven information about their efficacy.

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