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"A Review on Anticancer Activity of Vincristine"

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ABSTRACT

Vincristine and vinblastine were discovered inside the periwinkle leaf & plant. *Catharanthus roseus.* Vincristine acts as inhibition during the cell cycle's metaphase, inhibiting mitotic spindle improvement by binding to microtubules. Vincristine, a natural antimitotic compound, is a stable component in multiple cancer treatment regimens. Vincristine is appropriate for combination therapy.

Keywords: *Madagascar periwinkle*, Vincristine, Vinblastine, Tubulin, Cell Division, combinatorial treatment, cyclophosphamide,

INTRODUCTION

Cancer is a significant public health problem in both developing and industrialized nations. The ability of synthetic and natural or biologic and chemical substances to reverse, suppress, or protect carcinogenic progression is referred to as anticancer activity. A variety of synthetic agents used to treat the disease, but they are toxic. Herbal medicine has been reported to be safe and to have few or no side effects, especially when compared to synthetic drugs. Plants have always served as the foundation of traditional medicine systems, providing continuous remedies to humanity for thousands of years. Utilizing knowledge of medicinal plants for such preparation of different drugs has been extremely useful.[1] Medicinal plants are thought to be a rich source of a wide range of ingredients that can be used in drug development. Cancer is one of the most lethal diseases, characterised by irregular cellular proliferation. The most common cause of cancer is a change in lifestyle, and as a result, it has become a global issue all over the world. As a result, it is an urgent need for better treatments for this disease. Because radiation therapy and chemotherapy have a variety of side effects, using naturally occurring compounds may be an option.[2] Since ancient times, the use of natural plant medicines in Ayurvedic, a traditional Indian medicinal system, has proved beneficial in avoiding or suppressing a variety of tumours through several lines of therapy. [3] In India, citizens of various ethnic groups and geographical regions have their own unique cultures, religious practises, dietary habits, and a wealth of traditional medical knowledge. [4] They use herbal medicine to treat a wide range of illnesses. For thousands of years, different ailments have been treated using natural products, particularly plants.Ancient civilizations like Egypt, China, India, and Greece used terrestrial plants as medicine, and an impressive range of contemporary drugs have been created from them. About 2600 BC, the Sumerians and the Akkaidians produced the earliest written accounts of plant medicine. [5] 80% of people worldwide use traditional treatment methods, according to the World Health Organization (WHO).

Vinca alkaloid are naturally obtained from pink madagascar periwinkle, Catharanthus roseus G. Don. The flavonoids are crucial because they function as anticancer medications like vincristine.

The extremely high price of and vincristine is due to their extremely low productivity in plants (0.001-0.0003%). Horseradish peroxidase catalyses the coupling of vindoline as well as catharanthine to form vinblastine, a diametric indole alkaloid[6]. Very little (0.9%) coupling product yield was reported. By oxidising the methyl group on vinblastine, vincristine is created. Most of the important enzymes involved in the biosynthesis of indole alkaloids have been isolated from C. roseus seedlings and/or cell suspension cultures [7]. Catharanthine is generated in significant amounts in cell cultures, but diametric or monomeric indole alkaloids are not produced. In only shoot cultural contexts and differentiated tissues, not in roots, were vincristine, vinblastine, and vindoline reported. [8,9To achieve

industrialization of the alkaloids, C. roseus plant cell lines that are stable, highly productive, and salt tolerant have recently been created.

Their low yield is the main drawback of these medications in cell cultures. Pharmacologists and chemists are particularly interested in improving catharanthine development in C. roseus cultured cells because vinblastine can be made from catharanthine and vindoline in high yields, and vindoline is widely present in plants [10]. According to Moreno et cie. in 1981 [11], elicitors can also regulate the production of such alkaloids. Due to the low and vincristine components in the plants, metabolic technique for alternative methods of production was encouraged or made feasible [8,12]. Engineering methods, such as partial chemical synthesis[13], total chemical synthesis[16,17], or even chemical or enzymatic coupling[18] of available commercially catharanthine and vindoline, are helpful. [14,15].

HISTORY

The first scientists to isolate Vincristine from the Madagascar madagascar periwinkle were Robert Noble & Charles Thomas Beer. When vinblastine was first identified as a chemotherapeutics, it was ground up into a tea to assess its usefulness.

Table No 1: Chemical Data Of Vincristine

FORMULA	C46H56N4O10
Mol. Mass	824.958 g/mol

Table No 2: Pharmacokinetic Data Of Vincristine

METABOLISM	HEPATIC
HAIF LIFE	19 to 155 hours
EXCREATION	MOSTLY BILIARY AND 10% RENAL

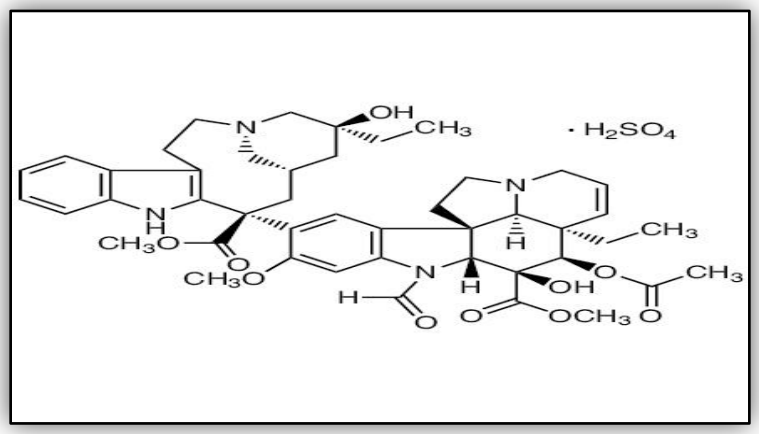

Fig No 1: Structure Of Vinecristine

According to research conducted in the 1950s, C. roseus contains more than 100 active ingredients, which is why it has been used as a natural remedy for centuries.

CLINICAL PHARMACOLOGY

Lack of sensitive assays for determining vincristine plasma concentrations limits pharmacokinetic studies of vincristine. Because of extensive tissue binding a & large volume of distribution, plasma clearance occurs quickly. Vincristine is believed to have tri-exponential pharmacokinetics, with rapid absorption after bolus injection, a 50–155 minute phase distribution, and an elimination half-life of approximately 85 hours. [21-25] Plasma clearance in kids seems to be higher than in adults. Cytochrome P450 3A breaks down vincristine in the liver, and medications taken at the same time may either inhibit or promote vincristine's clearance by this enzyme. [26,27]

Vincristine builds up in a variety of tissues, including the pancreas, spleen, lung, liver, kidney, and bone marrow. The adipose tissue, the eye, and the brain are largely exempt from it. [28] Vincristine is excreted in the bile and faeces as either the unchanged drug or as an metabolite. [29] Renal excretion of vincristine is not very high. It is not entirely clear how vincristine's antitumor effects relate to its plasma pharmacokinetics.

Adults typically receive vincristine as a bolus iv infusion at 1.4 mg/m2 (with a maximum dose of 2 mg), and children typically receive it at 1.5–2.0 mg/m2 . Vincristine may be administered up to once per week, but the dosing regimen varies depending on the type of cancer, the patient's response, and any other medications being taken concurrently. The dose-limiting toxicity is neurotoxicity, and attempts to reduce neurotoxicity by using continuous infusion rather than bolus injection have yielded mixed results. [30,31] When there is severe neurotoxicity or hepatic dysfunction, the dosages of vincristine is decreased. For moderate hyperbilirubinemia, patients typically receive 50% of the prescribed dose, and for severe hyperbilirubinemia, 25%. [32]

Side effects

Vincristine's dose-limiting adverse effect is neurotoxicity. Despite the fact that vincristine-induced neuropathy develops over time, some symptoms appear in the first weeks of treatment. Initial neurotoxin symptoms and signs include parenthesis and symmetrical sensory impairment. Later on, patients may lose their deep tendon reflexes, experience gross motor abnormalities like foot or wrist drop, or lose their fine motor skills like writing. Some patients experience autonomic polyneuropathy, which causes impotence, paralysis of the ileum, bladder dysfunction, & constipation. After therapy is stopped, many symptoms go away within a few weeks to months, but there may still be some residual neurotoxicity. [30,31]

The dose and administration frequency may have an impact on how severe the neurotoxicity is. Because of worries that autonomic neurotoxicity has more directly effected by the size of the single dose than by the sum of doses, vincristine doses are usually capped at 2 mg or 2.5 mg. [31,35,36]. The neurotoxicity caused by vincristine is most severe in newborns & the elderly, and this may be attributed to dose calculations. It has also been demonstrated that impaired vincristine biliary excretion is associated with neurotoxicity & obstructive liver disease. [36]

Vincristine's neurotoxicity may also get worse if radiotherapy or chemotherapeutic drugs like L-asparaginase are administered concurrently. [37]. Of note, patients who received high doses or who had their blood-brain barrier disrupted have experienced severe the central nervous system toxicity. Vincristine should never be administered intrathecally because it almost always results in death. [38]. Following vincristine administration, patients will also cause mild neutropenia and anaemia in addition to neurotoxicity. This is easily reversed, and treatment is typically not delayed as a result. [39] Depending on the dosage and length of the treatment, vincristine-related alopecia and rash can vary. Constipation, stomach cramps, nausea, and vomiting are typical gastrointestinal side effects. Patients may also express concerns about urinary symptoms as a result of polyuria, dysuria, or bladder rentention. [40]

MODE OF ACTION

The disruption of microtubules, inhibition of protein and nucleic acid synthesis, elevation of oxidised glutathione, modification of lipid metabolism and membrane lipid content, elevation of cyclic adenosine & inhibition of calcium-calmodulin controlled cAMP phosphodiesterase are just a few of the numerous biochemical effects observed after exposure of cells and tissues to vinca alkaloids. The Vinca alkaloids are highly hydrophobic molecules that, when left uncharged, partition into lipid bilayers, changing the composition and functionality of membranes. They have a variety of effects, but their only clearly defined direct action is the disturbance of microtubules, which comes about as a result of their reversible tubulin binding.The majority of vinca alkaloids' concentrations have pharmacological activity, and the biochemical effects of exposure to vinca alkaloids are likely secondary to microtubule disruption, it is possible that substance changes in bilayers may affect some membrane-dependent processes. These substances cause the formation of sizable crystalline aggregates made of tubulin and drug at high intracellular concentrations. The Vinca alkaloids have a variety of biochemical effects, but their anticancer activity is typically thought to be due to their ability to rupture microtubules, which results in the disintegration of mitotic spindles & meiotic division arrest through dividing cells. In addition to mitosis, microtubules have been involved in numerous other cellular processes, and exposed to vinca alkaloids results in a variety of pharmacological causes, many of which may affect crucial functions, in both the dividing & non deviding cells.

Vinblastine and vincristine treatment has been associated with morphological changes & cell death in non-dividing normal and leukemic lymphocytes, as well as in cultured leukemic cells in the interphase, G1 and S phases. Vinca alkaloids prevent cell migration in human monocytes & directional tumour cell migration in culture. Microtubules are

necessary for the motion of organelles along neuronal processes, including mitochondria & secretory granules, as well as the transportation of various metabolites. Vinca alkaloids cause neurotoxicity by preventing axonal transport in nervous tissue.The vinca alkaloids appear to interfere with membrane trafficking and disrupt the cytoskeleton in order to inhibit secretory processes. After receiving treatment with vinca alkaloids, platelets, which rely on the stability of a peripheral circle of microtubules for their propagate structure, develop a spherical shape. The vinca alkaloids exhibit a variety of possibly cytotoxic effects those are unconnected to mitotic inhibition, as these few examples show.

Although the effects of vinca alkaloids on the function and structure of microtubules have indeed been extensively characterised, methodological issues have made it challenging to determine the type & no of of vinca alkaloid binding on tubulin. However, it seems that each tubulin heterodimer has an undetermined number of nonspecific, low-affinity sites in addition to one "vincaspecific" site with a high intrinsic affinity. It is hard to compare a tubulin-binding properties of various vinca alkaloids because assay conditions and ligand-binding data analysis techniques vary. However, some generalisations are possible. [41]

Mechanism Of Action
Vincristine inhibits the development of microtubules by attaching to tubulin. Through disturbance of mitotic spindle forming, especially during in the M & S phases, this slowing mitosis to stop at metaphase. By preventing glycosyl utilisation, vincristine also interferes with the synthesis of proteins and nucleic acids. [42].

Administration
The practitioner or other healthcare provider always should examine the label for dosages, duration, and mode of administration before administering vincristine. Vincristine administration is never necessary in an emergency; however, vincristine administration done incorrectly or rapidly has resulted in several fatal cases.Vincristine should be infused intravenously over a brief period of 5 to 10 minutes. Vincristine should be administered in a 25 to 50 ml small bag, according to World Health Organization and the Center for Safe Medicines Practices (ISMP). Vincristine may be administered via a slow, 1-minute intravenous push if the mini bag is not readily available. Any other method of administration could be fatal. Vincristine needs to be given separately, not concurrently, to patients receiving medications designed for CNS administration. [43]

Being a vesicant, vincristine requires appropriate caution to ensure proper needle and catheter placement before and during iv infusion to prevent extravasation. Extravasation should be treated as an emergency and the inflow should be stopped right away, leaving the needle and cannula in place. Next, a gentle aspiration of the extravasated solution is necessary to prevent flushing of the line. The hyaluronidase remedy should then be started, the needle and cannula should be removed, and on days one and two of extravasation, a hot, dry compression must be applied for twenty minutes four times per day. Finally, reassessment should take place before the last dose of vincristine is given intravenously through a different vein. [44,45]

Metabolism
Vincristine rapidly leaves the blood system and is firmly obligated to tissues, it has a poor blood-brain barrier penetration rate. Vincristine is extensively metabolised by CYP3A4 in the liver. Patients who require dose adjustments have serum bilirubin levels greater than 3 mg/dl; the dose for these patients must be 50% of the usual dose, according to the manufacturer's label. Vincristine is mainly excreted in the faeces. [46]

Adverse Effect
Many bodily systems are impacted by vincristine's harmful side effects. Cardiovascular, Central nervous system, clinical, endocrine and metabolic, gastroenterological, gastrointestinal, hepatic, renal, breathing, neuromuscular, otic, & ophthalmic systems are a few cases of notable systems. Depending on the body system, vincristine administration may have some negative effects. [47]

Cardiovascular: Hypertension, ischemic heart disease, myocardial infarction, and edoema the brain and spinal cord Coma, diminished tendon reflex, paralysis, neuropathic pain, peripheral neuropathy, loss of sensation, vertigo, and motor dysfunction

Dermatology: Alopecia and rashes on the skin metabolic and endocrine: Weight loss, uric acid nephropathy, or hyperuricemia Constipation, nausea, vomiting, sore throat, or intestinal necrosis are gastrointestinal symptoms.

Genitourinary: Dysuria, urinary retention, or bladder dysfunction Other negative effects that are noteworthy might include:

Oncologic and hematologic: Leukopenia, mild thrombocytopenia, hemolytic uremic syndrome, anaemia, thrombotic thrombocytopenic purpura, and leukopenia.

Hepatic: Syndrome of hepatic sinusoidal obstruction

Local: Local annoyance
Neuromuscular & Skeletal: Jaw pain, amyotrophy, muscle aches, and back pain

Ophthalmology: Nystagmus, blindness, and optic atrophy Deafness, polyuria, bronchospasm, and dyspnea are adverse effects which may be less noticeable.

VINCRISTINE'S MECHANISM OF ACTION & SIDE EFFECTS

Vincristine and some other vinca alkaloids are considered to be mitotic poisons [48], especially tubulin-binding compounds, which have biological effects by impairing microtubule function. Tubulin heterodimers make up the polymeric fibres known as microtubules. The tubulin protein's - and -subunits combine to form dimers, and vincristine's binding site is found on the -subunit at the intersection of two heterodimers.

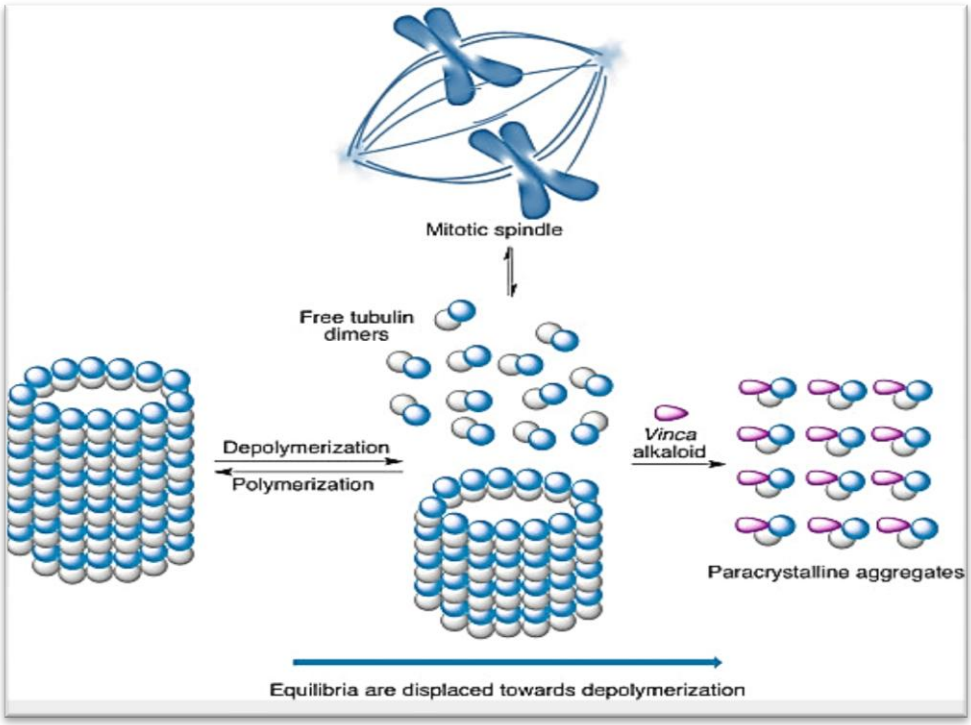

Fig No 2: Mechanism of Action

Thus, only tubulin-binding substances identified to date that do not tightly bind one microtubule heterodimer are vincristine and some other vinca alkaloids. [49] This significant characteristic is essential to the unique action mechanism of vinca alkaloids. The compounds are provides extensive of splitting the tubulin fibres at large doses. The fibres then come together and stay connected to one another thanks to the vinca alkaloid. In particular, a mitotic spindle, In particular, a mitotic spindle, w Vinca alkaloids, which bind the aims of the microtubule fibres and stabilise the dynamics of the microtubules, also impair this function at low doses. [51]

However, the antimitotic activity of vinca alkaloids does not only affect cancer cells. Consequently, there is a significant chance that vincristine therapy will result in serious side effects. Since they are related to the disruption of the cell cycle that affects not only cancer cells but also, regrettably, also healthy cells, the majority of the side effects brought on by vincristine are the same as those brought on by other antimitotic drugs. For instance, the intestinal mucosa houses one of these troubled cells. [52]. Additionally, vinca alkaloids-specific side effects do exist. The

myelosuppression is one of them. Reduction of WBC and red RBC, which is brought on by the disruption of progenitor cell proliferation, characterises this complicating condition.Because the blood do not have enough time to replenish between doses, this issue typically arises when chemotherapy is administrated at higher doses. Sepsis, bleeding, and other complications may develop as a result of the low blood cell levels. Vincristine-associated myelosuppression [a condition in which bone marrow activity is decreased, resulting in fewer red blood cells, white blood cells, and platelets.] is an unique condition that can occur, for instance, as a result of overdosing [53]. [54] Thrombocytosis, cellulitis (after extravasation), [55] thrombocytosis, [56] cellulitis (after extravasation), and other non-specific conditions like fever or skin rash[57] are among the undesirable side effects associated with vincristine therapy.

MEDICINAL USES

Traditionally, combination therapy regimens for medical treatments have included the vinca alkaloids. Since they operate through a different mechanism than drugs that alkylate DNA, they do not exhibit cross-resistance. [58] For both Hodgkin's and non-lymphomas, Hodgkin's as well as testicular carcinoma, VBL has been a crucial component of medical treatment regimens. [59] Additionally, it is applied to breast and germ cell tumours. White blood cell toxicity, nausea, vomiting, constipation, dyspnea, chest and tumour pain, wheezing, and fever are all side effects of VBL. Rarely is antidiuretic hormone secretion linked to it as well. [58]

Same as VBL, VRL. It can infect osteosarcoma cells and has significant anti - tumor activity in breast cancer patients. Additionally, VRL makes lipid bilayer membranes less stable. VRL has been authorised in the US for the initial management of patients with stage IV lung cancer. [60] Reduced reaction to infection, bruising and bleeding, anaemia, constipation, diarrhoea, nausea, tingling or numbness in the hands & feet, fatigue (also known as peripheral neuropathy), and swelling at the injection site are some of the side effects of VRL. Loss of hair and allergic reaction are less frequent side effects. [58]

Acute leukaemia, rhabdomyosarcoma, neuroblastoma, Wilm's tumour, Hodgkin's disease, and other lymphomas can all be treated with VCR. The ability of VCR to treat a number of non-malignant hematologic disorders, including hemolytic uremic syndrome, thrombotic thrombocytopenia purpura, and refractory autoimmune thrombocytopenia, has also been noted. The most frequent side effects of VCR include peripheral neuropathy, reduced bone marrow function, constipation, nervous system toxicity, nausea, and vomiting. [58,59]

VDS and VBL have comparable effects. Acute lymphocytic leukaemia, blast crisis of leukaemia, malignant melanoma, paediatric solid tumours, & metastatic renal, breast, esophageal, and colorectal carcinomas have all been linked to VDS's antineoplastic activity. [61]Vinflunine, a new artificial vinca alkaloid, was recently created by superacidic chemistry by adding two fluor molecules. [62] The first fluorocarbon microtubule inhibitor that is a member of the vinca alkaloids is vinflunine. This drug is being developed to treat other cancers and has been used Europe to treat 2nd transition phase cell carcinoma of a urothelium . It has been used for clinical research on a variety of solid tumours. Clinically, there has been significant progress, particularly in the management of transition phase cell carcinoma of a urothelial pathway, non-small cell cancer and breast cancer. Vinflunine also has been evaluated in patients with first-line advanced breast cancer and TCCU. [63]

ISOLATION AND DETECTION OF VINCA ALKOLOIDS

ISOLATION OF VINCA ALKOLOIDS FROM ENDOPHYTES ISOLATED FROM *CATHARANTHUS ROSEUS*

Endophytes:

An endophyte is a symbiotic microorganism, frequently a bacterium and fungus, that lives for at least a portion of its life cycle inside different parts of plants without obviously causing disease. They generate a variety of crucial secondary metabolites, such as cancer-preventive, anti-fungal, anti-diabetic, and immunosuppressive substances. Some of these chemicals are also made by the plants that serve as their hosts. A genetic recombination between the endophytes and the host may be the cause of some endophytes producing specific phytochemicals that were once unique to the host. Even a fungus that makes biosurfactants, Pseudozyma Antarctica, lives as an endophyte but was initially thought to be isolated from Antarctica. It generates the anti-cancerous glycolipid biosurfactant Mannosylerythritol lipid. [64]

Catharanthus roseus

The secondary metabolites of Catharanthus roseus may one day be used to treat a variety of diseases. The most parmacologically effective chemical components of Catharanthus roseus are alkaloids. They work by attaching to tubulin and preventing the formation of the mitotic spindle, which inhibits mitosis and ultimately results in metaphase arrest & cell death. [65]

Fig No.3: *Catharanthus roseus* plant

Ajmalicine, vinceine, vincamine, raubasin, reserpine, and catharanthine are present in roots and the basal stem, whereas vinblastin, vincrestine, vindesine, and vindeline tabersonine are primarily found in aerial parts. Anthocyanin pigment called rosindin can be discovered in the flower of the extraction and detection of vincristine and vinblastine were the main topics of this article. These two anticancer medications rank as the second best in the world.

MATERIALS AND METHODS

Sample collection, surface sterilization, culturing and isolation of endophytes
1. Sample collection: A catheranthus roseus leaf, fresh and healthy, was procured and placed in a sterile resealable plastic bag.

2. Surface sterilisation: The fresh leaf stem and root were washed in slow-moving yap water for a few minutes, then with a few drops of sterile water, twice or three times, and then with a solution of 0.1% mercuric chloride for 60 seconds. The process was then repeated several times with sterile water.

3. Growing endophytes: PDA (Potato Dextrose Agar) and NA (Nutrient Agar) media were made, and both were autoclaved for 15 minutes at 121°C and 15 psi. The leaves & stem were then cut into pieces using sterile forceps and scarpels, and 20 ml of PDA & NA were added aseptically. The mixture was then allowed to solidify at room temperature. The plates then are incubated for 10 to 12 days at room temperature. On the edges and sides of the petri dish, there was bacterial and fungal growth. The top six of them were chosen and incubated once more for pure culture.
Catharanthus roseus mature fresh healthy leaves were collected in a sterile polythene bag. Freshly collected leaves' stems and roots were washed in 2-3 drops of water after being slowly run under the faucet for a few minutes. Then thoroughly washed for two to three times with sterile water. Now, these were washed thoroughly with distilled water before being rinsed for almost 60 seconds with 0.1% mercuric chloride. The media for PDA andNA were prepared & inoculated at 121°C at 15 psi for 15 min. 20 ml each of sterilised PDA and NA were aseptically poured into pre-sterilized glass petriplates, where they were left to solidify at room temperature.The leaves and stem were minced, and the leaves were also macerated, using a sterile scalpel & forceps. The plates were incubated for almost 10-15 day at room temperature, during which time bacterial and fungal growth was seen on the media as well as on edges of leaves, stems, and macerated leaves.

Six desirable endophytic colonies were chosen from among the many endophytes, and their corresponding pure cultures were created. Then, the bacterial isolates were kept at room temperature for incubation. Stock cultures were sub-cultured and kept at 40°C. For fungal growth, PDA medium is preferred, while NA is preferred for growth of bacteria.

Fermentation:-
Endophyte fermentation takes place out on pure culture and occurs in two stages.

Stage 1: Making the Maltose, Glucose, and Yeast, peptone [MGYP] extract Endophytes were used as the seed culture and were inoculated with a 7-day-old culture in to the 100 ml of MGYP media in a 500-ml Erlenmeyer flask. They were then incubated at 28°C on the mechanical shaker (150 rpm) for 4-5 days.

Stage 2: Vinca medium, a production medium, was inoculated with 10 ml aliquots from Step 1 flasks. Vinca media (1000 ml) contains the following ingredients: Glucose: 30 g, Sodium benzoate: 100 g, Peptone: 1 g, Magnesium sulphate: 3.6 mg, Biotin: 1 mg, Thiamine: 1 g, Pyridoxal: 1 mg, Calcium pentothenate: 1 mg, Phosphate buffer: 1 ml (pH 6.8), L-Tryptophan-0.01g, Geranium oil - 0.005g.

These were incubated at 280C.This is carried out for 20 days

EXTEARTION OF VINCA ALKOLOIDS

Extraction and purification of alkaloids:-
1. A dry vinca leaf is extracted to 0.1 ml HCI for 30 minutes in an ultrasonic bath, and the mixture is centrifuged for 10 minutes.
2. Filtering the sediment after it has been re-extracted with more hcl and mixed with the supernatant.
3. Apply petroleum ether to this fluid to get rid of chlorophyll and lipophilic compounds.
4. Sort the acidic fraction and use an alkaline solution to treat it. To form the precipitate, slowly add 10% embonic acid.
5. Raise the pH to 5 and use decantation to separate the precipitate for vincristin semisynthesis.
6. Combine this crystalline with 0.1M HCL & 0.1M citric acid, then use dichloromethane and an ice bucket to cool this from 0 to -5 °C.
7. Slowly add 1% sodium borohydride solution in methanol, 10% aq hypochlorite, and 30% aq hydrogen peroxide for 3 to 5 hours. 8. Boost the pH to 9.5. 9. Gradually gather and dry the organic layer.

Verify for vinca alkaloids' presence.
By using tried-and-true techniques, the extract, which primarily contained vincristine and vinblastine alkaloids, was discovered.

1. The Mayer Test
Alkaloids can be found in natural products using Mayer's reagent, an alkaloidal precipitating reagent. Fresh Mayer's reagent is made by dissolving a solution of potassium iodide (5.00 g) and mercuric chloride (1.36 g) in water (100.0 ml). A test tube was filled with 3ml of the extract. Mayer's reagent was added in small amounts along the test tube's sides.

2. **Picric acid Test:**
Picric acid test is carried out by using Hager's reagent. Hager's reagent is prepared by adding 1g of picric acidin 100 ml distilled water. 3ml of extract was taken in a test tube. Few drops of Hager's reagent was added along sides of test tube.

***3. High Performance Liquid Chromatography (HPLC*)**
To separate, quantify, and analyse the elements from the compound mixture, HPLC is used. Use is made of the SPD-M20A prominence detector with diode array coupled to the C-18 symmetry column (Enable). The highest level of sensitivity and stability available in a PDA detector today is offered by SPD-M20A high-performance liquid chromatography PDA detector. Use of 5-95% acetonitrile in water to 0.01% trifluoroacetic acid and a flow rate of 0.5 ml/min was used to carry out the gradient elution. The substance was found using a wavelength recorder set to a 200–300 nm range. One milligramme of normal vincristine (Sigma-Aldrich) was dissolved in one millilitre of acetonitrile (Himedia) to create a stock solution.(Conc.1mg/ml). From the stock solution, various dilutions with various numbers were made. These standards have been injected into an HPLC column for 30 microliters, and their chromatograms have been recorded. Alkaloids were extracted with the aid of ethyl acetate from of the culture filtrate that was obtained after 15 days. Acetonitrile of HPLC grade was used to dissolve the purified endophytic vincristine. On the calibration curve produced by the various dilutions of the standard, the RT values & the area under the point evaluation for the extracts were noted. [68]

Hepatotoxic effect of sub-acute vincristine
Animals and experimental design
The animal house of the Science College obtained and cared for healthy adult male Wister rats (weight: 180–250 g). Four rats were housed in each cage with such a 12-hour light/dark cycle during adaptation and the experimental procedure in such a temp-controlled room (25 C). Water and everyday food were available to them without charge. The

Institutional Animal Ethics Committee, which follows the standards established for the treatment and use of laboratory animals published by the US National Institute of Health, gave its approval to all procedures. The animals underwent a one-week adaptation period before being divided into 5 groups (n = 6/group) and receiving the following care for 30 days:

(1) The control group received normal saline (0.9% NaCl, i.p.);
(2) The VCR-treated group received VCR as VCR-sulfate at a final dose of 50 lg/kg, i.p.;
(3) The VCR-Broccoli treated group received concurrent doses of VCR-sulfate (50 lg/kg, i.p.) & broccoli aqueous extract (200 mg/kg, orally);
(4) VCR B. juncea (mustard)-treated group, received concurrent doses of VCR-sulfate (50 lg/kg, i.p.
(5) VCR Broccoli B. juncea-treated group, administrated concurrent dose of the VCR-sulfate (50 lg/kg i.p.), B. juncea, & broccoli extracts, as described above.

The dose was the lowest effective dose required to induce destruction in healthy tissue, such as neuropathic pain, after the simple term (ten days) administration to rats. The dose & route of VCR-sulfate administering were chosen based on prior studies. The doses of broccoli or the methods of administration, however, were chosen based on a number of studies that showed how these extracts could be used safely and had strong antioxidant & hepatoprotective effects in rats when compared to doses used in other animal models. [69,70]

Blood and tissue collection

On the last day of treatment, all rats underwent sodium pentobarbital anaesthesia (60–70 mg/kg, i.p.) after being developed for up to 12 hrs. For the purpose of determining the levels of hepatic enzymes and performing biochemical analysis, blood samples from the heart & sera were taken and separated. The livers were then quickly removed after all animals were put to death. In accordance with the kit manufacturers' recommendations, portions of the livers were immediately frozen at 80 C, homogenised in the appropriate buffers, and the supernatants were obtained for use in the ensuing biochemical analysis of the oxidative stress parameters. Reverse transcription chain reaction was carried out on the remaining components, which were obtained in RNA stabilising reagent and kept at 80 C. (RT-PCR). Additionally, additional liver tissue was corrected in 10% formalin soln &used later for histological analysis.

Combinations of Vincristine with Cyclophosphamide, Doxorubicin, and Prednisone

The "CHOP" regimen, which combines vincristine with doxorubicin hydrochloride (Oncovin, Eli Lilly and Company, Indianapolis, IN, USA), cyclophosphamide (Cytoxan, developed by AstaWerke Aktiengesellschaft Chemische Fabrik, Brackwede, Germany), and prednisone or prednisolone, is likely the most well-known drug combination that contains vincristine. Non-Hodgkin lymphoma has traditionally been treated with a combination of the medications listed. [71].This combination primarily targets DNA in its cytotoxic action. The popularly used alkylating agent cyclophosphamide, which received approval in 1959, successfully induces DNA cross-linking. [72] Doxorubicin then interacts into the DNA & binds proteins essential for transcription and replication of DNA. Inhibiting DNA, RNA, & protein synthesis and ultimately initiating cell death. [73] This action led to the FDA's 1974 approval of doxorubicin for the diagnosis of soft-tissue sarcoma. [74]. In addition to the cytotoxic medications mentioned above, the CHOP regimen also includes the administration of the corticosteroids prednisone & prednisolone because of their anti-inflammatory effects. [75]

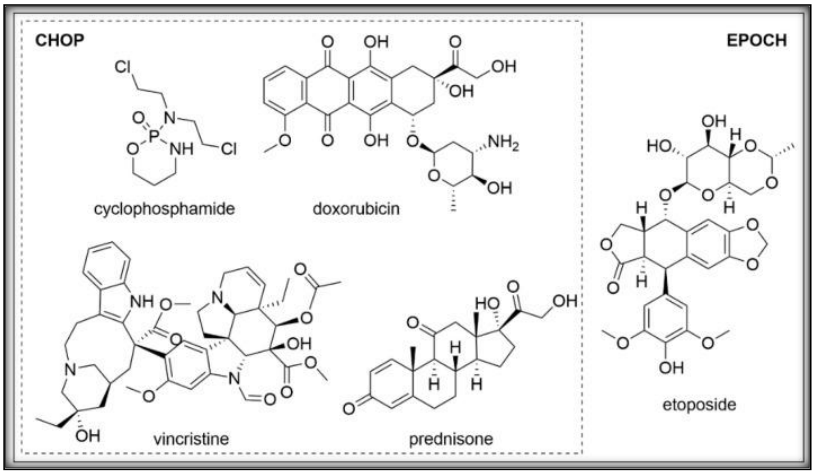

Fig No 4: Combinations of Vincristine with Cyclophosphamide,

Doxorubicin, and Prednisone

At the moment, CHOP is more frequently combined with rituximab (R-CHOP). Rituximab (Rituxan), a chimeric anti-CD20 IgG1 monoclonal antibody created by Biogen, Cambridge, Massachusetts, USA. The cell surface protein CD20, which is its target, is crucial for the growth and differentiation of B cells. [77] In 1997, the FDA approved rituximab alone for the management of B cell non-Hodgkin lymphoma indolent forms. Nine years later, the combination of rituximab and CHOP was approved. [78] Although the effectiveness of the CHOP regimen is significantly improved by the addition of rituximab, about one-third of sick people still experience relapses. [79Thereby, this combination is presently being tested in clinical trials along with other medications for treatment of various cancers. Etoposide is one of the commonly used medications when CHOP or R-CHOP is being used (Vepesid, Bristol-Myers Squibb, New York, NY, USA). The FDA gave this medication approval in 1983, and it works by interacting to topoisomerase II, specifically with the ligation action of this crucial enzyme that maintains DNA topology. As a result, after receiving etoposide treatment, cells produce a greater amount of cleaved DNA. [80] R-CHOPE, or even more commonly EPOCH-R, is the name given to the compound formed when etoposide is combined with R-CHOP. It seems to work well in some specific aggressive B-cell lymphomas that don't respond well to R-CHOP alone, like lymphomas caused by c-myc gene rearrangements. Dose-adjusted EPOCH-R induces long-lasting remission in patients with the cmyc rearrangement, as demonstrated in a potential, multicenter, singlearm phase 2 study. [81] EPOCH-R may aid in the treatment of Burkitt lymphoma in a manner similar to a lymphoma with c-myc rearrangement. Currently, CHOP and methotrexate are used in intensive combination regimens to treat this agressive of B cell lymphoma, which is common in children. [82] Unfortunately, because combinations to methotrexate are frequently toxic, these regimens, which have been created primarily for children, are not suitable for adult patients. This is especially true for those who have comorbidities, such as HIV. Nevertheless, EPOCH-R appears to be ineffective in these patients to be effective, as confirmed in a multicenter study [83]. EPOCH-R may be used to treat primary mediastinal big B cell lymphoma in addition to the previously mentioned types of lymphoma. Although the efficiency of EPOCH-R & R-CHOP against such a cancer are similar, R-CHOP is typically combined to radiotherapy in this instance in clinical practise. EPOCH-R is extremely effective in Shah et almulticenter.'s analysis also without radiation therapy, which would be an significant benefit of such treatment. [84]

CONCLUSIONS

Anticancer medications vincristine work by attaching to intracellular tubulin. Vincristine prevent DNA repair and RNA synthesis in tumour cells, which prevents the DNA-dependent & RNA Polymerase from working. *Catharanthus roseus* leaves were used to extract the vinca alkaloids, & their presence was confirmed. From the leaves, which were then cultured on PDA and NA media, the endophytes were isolated. Through microscopic examination, the isolated endophytes represent the desired morphological characteristics. Multiple stage fermentation was done using the prepared pure cultures of such endophytes. The endophytes of *C. roseus* showed the greatest potential for the production of vincristine quantitative and qualitative analyses.

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