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SYNERGETIC AND ANTIGONISTIC EFFECT OF ENRICHED PAPAYA LEAF EXTRACT AGAINST CISPLATINS INDUCED IN MCF-7 CANCER CELL LINES

Surekha Agraharam[1], Aruna Kumari Mullangi[2], Obaiah Jamakala[3], Kutagolla Peera[4*]

[1] Lecturer in Botany, Department of Botany, Sri Venkateswara Arts College, Tirupati-517502, Andhra Pradesh, India.

[2] Lecturer in Botany, Department of Botany, Government Degree College, Nagari, Chittoor Dist. Andhra Pradesh, India.

[3] Department of Zoology, Sri Venkateswara University, Tirupati-517502, Andhra Pradesh, India.

[4]Department of Zoology & DBT-BIF Centre, Sri Venkateswara University, Tirupati-517502, Andhra Pradesh, India.

ABSTRACT:

The present study aimed to prove that enriched papaya extract as an anti-thrombocytopenic drug, synergetic and antagonistic effect on efficacy of cisplatin against MCF-7 cell progression. MTT test was carried out to test the cytotoxicity of enriched papaya extract against the MCF-7 cell lines. Inhibitory efficacy of enriched papaya extract alone and in presence of cisplatin on MCF-7 cells was observed to analyze the antagonistic and/synergetic effect. DAPI, PI and Green fluorescence strains were used to observe the morphology and nucleus of the cells. BAD gene, an proapoptotic gene expression (qualitative) was carried out by using PCR and agarose gel electrophoresis to observe its gene expression in untreated, treatment with cisplatin and papaya extract in MCF-7 cell lines. The present study revealed that papaya extract don't exhibit significant inhibition/progression against MCF-7 Cells. Even it did not affect the efficacy of cisplatin against MCF-7 cell progression. The PCR results revealed that BAD genes expression was happened in cisplatin and papaya + cisplatin treated and did not get clear bands in untreated and papaya alone treated cells. Thus concluding that enriched papaya extract might be useful in management of thrombocytopenia induced by cisplatin without any interactions.

Key words: Cancer, Papaya Leaf Extract, MCF-7 Cell Line, Electrophoresis**,** MTT assay, Antagonistic effect.

INTRODUCTION

The word 'cancer' emanates from the Greek word 'karkinos' meaning crab. Hippocrates, the Greek physician (460-370 B.C.) gave this name because the tumors that are the visible evidence of cellular mass in many types of cancer like a crab with a central body (the tumor or lump) from which several rays-the legs, spread into the surrounding tissue (Moss, 2004; Hajdu, 2010). It is a genetic disorder involving dynamic changes in the genome leading to uncontrolled cell growth, ability to invade and metastasize. Cancer is characterized by uncontrolled, abnormal growth of cells (proliferation). The first historical description of

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this condition was related to breast carcinoma (Hippocrates), but malignant cancer masses also called malignant tumors have been found Egyptian mummies, about 5000 years old (Hajdu, 2010) . The term 'Neoplasia' describes a new tissue or cell growth, mostly from the own-body-cells except chorio carcinoma, which develops from embryonant placental tissue.

Cancer is the most progressive and divesting disease posing a threat of mortality to the entire world. The prevalence of cancer in India is estimated to be around 2.5 million, with about 8 lakhs new cases and 5.5 lakhs deaths per annum (Nandakumar, 2005). The burden of cervical cancer in India is enormous accounting for about 20% of all cancer related deaths in women and is the main cause of death in the middle age Indian women. It is estimated that there will be 16 million new cases by the year of 2020 (Anant Narayan *et al.,* 2010). It is believed that in near future the number of cancer patients will increase in the developing and under developed countries. Though the rate of cancer frequency in India is less than that of the western countries but due to the large population size, number of cases is more prevalent at any time (Krishnan and Sankaranarayana, 1991).

The cancer cell is characterized by acceleration of cell cycle, genomic alterations, invasive growth, increased cell mobility, chemotaxis changes in the cellular surfaces and secretion of lytic factors. Morphologically the cancerour cell is characterized by a large nucleus, having an irregular size and shape with prominent nuclei. The nucleus of neoplastic cells plays a major role in the assessment of tumor malignancy. Changes include its surface, volume, the nucleus/cytoplasm ratio, shape and density, as well as structure and homogeneity. These morphological and nuclear characteristics reflect the changes occurring at metabolic level, with the augmentation of structures in relation to cell division and the attenuation of structures associated to other metabolisms (Cooper et al., 1985; Failkow, 1979).

The cytoplasm also undergoes changes which includes the alteration of some surface proteins. Accumulation of ribosomal and messenger RNA in the cytoplasm makes it basophilic. Malignant cells have a small cytoplasmic amount, frequently with vacuoles. Degenerative cellular changes can be expressed by cytoplasmic inclusions. In some of neoplasms, apoptosis occurs with presence of apoptotic bodies. Epithelial carcinomas contain cytokeratins, mesenchymal tumours contain vimentin and in the central nervous system cells is an acid protein from glial, with a special role in tumour diagnosis (Kallergi *et al.,* 2011; Hynes, 1976).

Malignant cells change their enzyme content, such as the reduction of acid alkaline phosphatase. Changes occur in relation between sugars and the sialic acid from glycolipids and glycoproteins and also the negative loading of the cell surface. The plasma membrane of malignant cell favors accelerated transport of nutritive substances, especially sugars and amino acids. The surface of malignant cells displays differentiation antigens that express a normal development of the cancerous cell and antigens specific for the tumor, which appear with the oncogenic transformation. These receptors in malignant cells are altered and which modifies the cell agglutination behavior. On the surface of malignant cells, atypical

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microvilli, pseudopods vesicles cell surface enzymes were constitutively expressed (Mareel and De Mets, 1984; Hynes, 1976).

The functional changes of neoplastic cells cause the formation and elimination of active substances like growth factors, hormones and some lytic enzymes (collagenase, cathepsin and plasmogen activator), which favors the increased mobility and dissemination of neoplastic cells (Sporn and Roberts, 1988). Major alterations occur in energy metabolism, between normal and malignant cells especially the use of glucose. Cancerous cells exhibit anomalies of both glycolysis and the tricarboxylic acid (TCA) cycle. The cancerous cell is particularly characterized by the usage of limited oxygen and the massive use of glucose (Evan and Vousaden, 2001). Based on the physiological and molecular functions a neoplasia can be benign or malignant.

Breast cancer is the most common cause of cancer in women and the second most common cause of cancer death in women in the u.s. Breast cancer refers to cancers originating from breast Tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts milk. world wide, breast cancer comprises 10.4% of all cancer incidences amon women,making it the second most common type of non-skin cancer(after lung cancer)and the fifth most common cause of cancer death in 2004, breast cancer caused 519,000 deaths world wide(7% of cancer deaths; almost 1% of all deaths) (Ganesh N. Sharma 2010). Over the last 10 years or so,breast cancer has been rising steadily, and for the first time in 2012,breast cancer was the most common cancer in womenin india,a way head of cervical cancer.(ferlay j et al.,2012).This is partly due to an actual decrease in the incidence of cervical cancer.However,mostly due to a rapid rise in the number of breast cancer cases,the incidence of this disease has been consistently increasing,and it is estimated that it has risen by 50% between 1965 and 1985.the annual percentage change in the incidence ranged from 0.46 to 2.56 for breast cancer.in 2015, there will be an estimated 155,000 new cases of breast cancer and about 76,000 women in india are expected to die of the disease(Asthana,s et al.,2014). Breast cancer is about 100 times more common in women than in men ,although males tend to have poorer outcomes due to delays in diagnosis.cancer cells are very similar to cells of the organism from which they originated and have similar (but not identical) DNA and RNA. (Ganesh N. Sharma 2010).

Cancer cells are formed from normal cells due to a modoification/mutation of DNA and / or RNA. These modifications / mutations can occur spontaneously ill law of Thermodynamics-increase of entropy or they may be induced by other factors such as;nuclear radiation , electromagnetic radiation(microwaves,X-rays,Gamma-rays ,ultraviolet-rays etc.),viruses,bacteria and fungi, parasites due to tissue infla,mation /irritation,heat,chemicals in the air,water and food ,mechanical cell-level injury, free radicals,zvolution and ageing of DNA and RNS ,etc. All these can produce mutations that may start cancer . cancer can be called therefore “Entropic Disease”since it is associated with the increase of entropy of the oraganism to the point where the organism cannot correct this itself external intervention is required to allow the organism to return to a stable entropic state(Mieszkowski M.R. cancer 2010).

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Cancer develops if the immune system is not working properly and/or the amount of cells produced is too greater for the immune system to eliminate(K.K Sharma 2010).The rate of DNA and RNA mutations can be too high under some conditions such as;unhealthy environment (due to radiation, chemicals,etc) (Helmberg A.2010),poor diet (unhealthy cell environment)(k.k Sharma , 2010).people with genetic predisposition to mutations and people of advanced age(above 80)(Ershler W.B ,2005).

Usually, cancer is named after the body part in which it originated ; thus , breast cancer refers to the erratic growth and proliferation of cells that originate in the breast tissue (Khuwaja G.A., 2004).the breast is composed of two main types of tissue that is glandular tissues and stromal tissues.glandular tissues hows the milk producing glands (lobules)and the ducts (the milk passages)while stromal tissues include fatty and fibrous connective tissues.the breast is also made of lymphotic tissue –immune system tissue that removes cellular fluids and waste (piush Sharma,2010).

There are several types of tumors that may develop with in different areas of breast. Most tumors are the result of benign changes within the breast, the most breast cancers begin in the cells that line the ducts . some begin in the cells that line the lobules , while a small number start in the other tissue(Rahuldev,2010).

Carcinogenesis and Malignant Transformation

Carcinogenesis is the process whereby cells with a growth advantage over their neighbors are transformed by mutations in the genes that control cell division into cells that no longer respond to regulatory signals. Carcinogenesis is a Multi-stage theory in which the malignancy arises from a transformation of the genetic material of a normal cell, followed by successive mutations, ultimately leading to the uncontrolled proliferation of progeny cells (David and Landolph 2005). The overall process includes a three stage model, initially a permanent change was occur in the genetic material (one somatic cell), secondly, the mutated cell clone was the expanded (promotion) and finally, the malignant conversion into cancer (progression) (Fig 1). The damage can be caused by chemicals, physical agents, virus or bacterial infections, the so called carcinogenic agents (Barnes *et al.,* 2018).

Tumor Initiation

In the initial step of the cancer development, a permanent change in genetic material of one cell is achieved by DNA mutation. DNA mutation triggered either by biological factors includes viruses and bacteria, radiation and chemicals which can damage the DNA through hydroxyl radicals (OH) (Chang and Parsonnet 2010; Dizdaroglu *et al.,* 2002; Zur Hausen, 2002). Increased oxidative stress, which may in turn deplete the endogenous antioxidant reserves, is an important signal leading to Ca2+ mobilization. Reactive oxygen species (ROS) mediated Ca2+ changes leads to the activation of endonucleases which can cause DNA fragmentation during apoptosis (Gilberto *et al.,* 2004).

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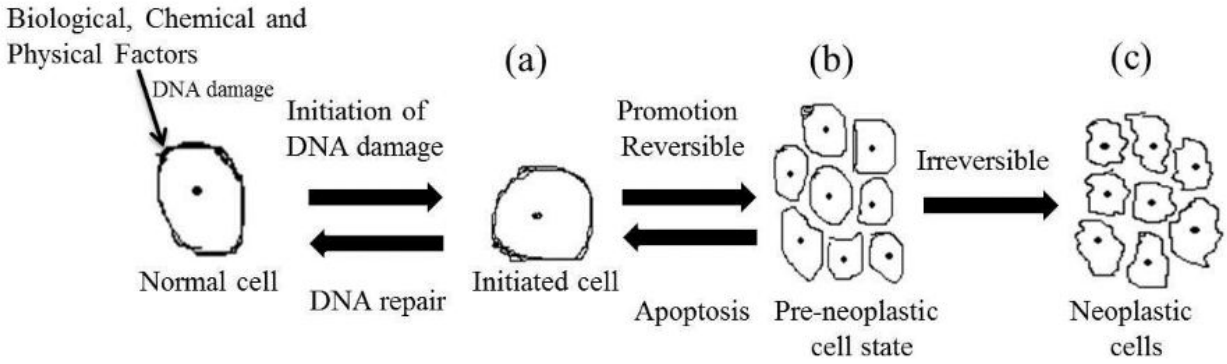

Figure 1: Three stage model of carcinogenesis shows a) Initiation (Biological, Chemical and Physiological factors), accumulation of carcinogenic mutations; b) progresses through pre-neoplastic (Reversible) stages by the acquisition of more mutations and up regulated cell signalling; c) progression by a tumour promoters and development of angiogenic potential leading to the expression of neoplastic stage which is irreversible condition. A series of DNA damage was caused by a variety of mechanisms. DNA damages include sugars and base modifications, strand breaks and DNA protein crosses links. Modified DNA base (pyramidine and purine) constitute one of the most lesions which has mutagenic properties being potentially able to damage the genome integrity (Wallace, 1998). One of the most studied DNA damaged by radiation and chemical carcinogen (8-oxoguanine) which can modify the DNA bases leading to GC-TA transversion mutagenesis (Gilberto *et al.,* 2004; Grollman and Moriya, 1993). On the other hand the modified DNA activates the proto-oncogene to oncogene. As a result of modification, oncogene gets produce an altered version of functional protein with over expression and the result is an uncontrolled growth/cell proliferation. Several modified bases which have also been shown to possess miscoding potentials and thus perhaps pre-mutagenic properties (VanderVeen *et al.,* 2000). Armitage and Doll, (1954) also hypothesized that cancer incidence should be proportional to the concentration of the effective carcinogen. However, experimental data suggests that tumor incidence and concentration of the carcinogen vary in proportion.

Tumor Promotion and Progression

After the initiation, the next step in carcinogenesis is the promotion, which indicates that the genome damage, set in the initiation step, could not be repaired and it is now transferred to or multiplied to daughter cells. A number of tumour promoters are thought to act either by stimulating endogenous molecules produced by altering cellular metabolic processes (Gilberto *et al.,* 2004). Cancer promoting agents are similar to carcinogens, which increasing the cell growth by causing an alteration of cellular interactions, components important for controlled cell growth.

One of the most studied cancer promoting agent was phorbol esters which activates protein kinase C, (PKC) a cellular signal transduction protein and proto-oncogene which can initiates and promotes the carcinogenesis by a stimulation of the enzyme ornithin decarboxylase, necessary for the mitogenesis (Grollman and Moriya, 1993; McCann & Pegg 1992). Hence, the stimulation of intracellular molecules is considered the main way to

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promote the chemical carcinogenesis. In *in vivo* experiments of Fehrmann *et al.* (2003) reported that, the binding of HPV viral E6 protein to p53, AP-1 and E7 protein to PRb were over activated. The binding results in the E6-AP-mediated ubiquitation and rapid proteosomal degradation of p53 and the binding disrupts the complex between pRb and the cellular transcription factor E2F, resulting in the liberation of E2F, which allows the cell to enter the S-phase of the cell cycle (Von Knebel *et al.,* 1992; Munger *et al.,* 1989).

The Progression of carcinogenesis comprises the acquisition of malignant properties to the tumor cell. Progression is characterized by accelerate cell proliferation, escape from immune surveillance, tissue invasion and metastatis (Toyokuni *et al.,* 1995). This tumour has all the characteristics feature mentioned in the malignant neoplasia. Factors like immune system response, localization, blood support and the rates of growth, play an important role in the progression. During the cancerogenesis process, cancer cells exhibits new or modified antigens on the outer cell membrane. These antigens are cell type- tissue and species, specific molecules. Since the generation of large amounts of promoters may attribute to the ability of some tumours to transform (mutate), inhibit antiproteases and damage local tissues, it has together with the increase in the level of modified DNA bases (Malins *et al.,* 1996). Conversely, the increased levels of modified DNA bases may contribute to the genetic instability and metastatic potential of the tumour cells in fully developed cancers cells (Schmielau and Finn, 2001).

Metastasis

Cancer cells spread locally or through the bloodstream and lymphatic system to another parts of the body, depending on type of cancer and location. Like most other cellular mechanisms, the metastasis seems to be initiated by an activation of genes (Raymond, 2007).

Proliferation and migration of neoplastic cells from a tumor is unpredictable. The movement of neoplastic cells starts with the formation of irregular cytoplasmic pseudopods, which infiltrate through basal membranes (Cooper and Hausman, 2000). The active locomotion of malignant cell involves the enzymatic dissolution of the surrounding host tissue, especially intestinal matrix. At the beginning of invasion, loosening intestinal matrix of the host tissue occurs, by the appearance of an edema. The size of the interstitial fluid volume facilitates cell locomotion. The destruction of the host tissue, with the invasive growth was caused by enzymatic process and partially by atropy through the pressure exerted by the tumor tissue (El-Metwally and Tarek, 2009).

Major types of cancers

The major types of cancer are carcinoma, sarcoma, melanoma, lymphoma, and leukemia.

- Carcinomas the most commonly diagnosed cancers originate in the skin, lungs, breasts, pancreas, and other organs and glands.
- Sarcomas arise in bone, muscle, fat, blood vessels, cartilage, or other soft or connective tissues of the body. They are relatively uncommon.
- Melanomas are cancers that arise in the cells that make the pigment in skin.
- Lymphomas are cancers of lymphocytes.

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- Leukemia is cancer of the blood. It does not usually form solid tumors.

Types of cancer treatments

Chemotherapy, surgery and radiotherapy are the most common types of cancer treatments available nowadays. The history of chemotherapy began in the early 20th century, but its use in treating cancer began in the 1930s. The term "chemotherapy" was coined by the German scientist Paul Ehrlich, who had a particular interest in alkylating agents and who came up with the term to describe the chemical treatment of disease. During the First and Second World Wars, it was noticed that soldiers exposed to mustard gas experienced decreased levels of leukocytes. This led to the use of nitrogen mustard as the first chemotherapy agent to treat lymphomas, a treatment used by Gilman in 1943. In the following years, alkylating drugs such as cyclophosphamide and chlorambucil were synthesized to fight cancer (Gilman, 1946; Goodman et al., 1946). Kilte and Farber designed folate antagonists such as aminopterin and amethopterin, leading to the development of methotrexate, which in 1948 achieved leukemia remission in children (Farber et al., 1948). Elion and Hitchings developed 6-thioquanine and 6-mercaptopurine in 1951 for treating leukemia (Hitchings et al., 1954; Elion et al., 1954). Heidelberger developed a drug for solid tumors, 5-fluorouracil (5-FU), which is up to now an important chemotherapy agent against colorectal, head and neck cancer (Heidelberger et al., 1957). The 1950s saw the design of corticosteroids, along with the establishment of the Cancer Chemotherapy National Service Center in 1955, whose purpose was to test cancer drugs. At that time, monotherapy drugs only achieved brief responses in some types of cancers (Pearson et al., 1949). By 1958, the first cancer to be cured with chemotherapy, choriocarcinoma, was reported (Li et al., 1958). During the 1960s, the main targets were hematologic cancers. Better treatments were developed, with alkaloids from vinca and ibenzmethyzin (procarbazine) applied to leukemia and Hodgkin's disease (Johnson et al., 1963; Brunner et al., 1965; DeVita et al., 1966). In the 1970s, advanced Hodgkin's disease was made curable with chemotherapy using the MOMP protocol (De Vita et al., 1965; Moxley et al., 1967), which combined nitrogen mustard with vincristine, methotrexate and prednisone, and the MOPP protocol (De Vita et al., 1967; De Vita et al., 1970), containing procarbazine but no methotrexate. Patients with diffuse large B-cell lymphoma were treated with the same therapy and, in 1975, a cure for advanced diffuse large B-cell lymphoma was reported using protocol C-MOPP, which substituted cyclophosphamide for nitrogen mustard (DeVita et al., 1975).

Surgery and radiotherapy

Surgery and radiotherapy were the basis for solid tumor treatment into the 1960s. This led to a plateau in curability rates due to uncontrolled micro-metastases. There were some promising publications about the use of adjuvant chemotherapy after radiotherapy or surgery in curing patients with advanced cancer. Breast cancer was the first type of disease in which positive results with adjuvant therapy were obtained, and also the first example of multimodality treatment, a strategy 3281 currently employed for treatment of numerous types of tumors.

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Chemotherapy

In the late 1960s, the use of adjuvant chemotherapy changed the concept of localized treatment. There was significant progress in 1978 when higher cure rates of metastatic germ cancer were achieved by combining cisplatin, bleomycin and vinblastine (Einhorn et al., 1979; Einhorn et al., 1977; Einhorn, 1981). The experience with polychemotherapy in hematologic cancer brought to light the fact that different drugs act against tumor cells in different phases of their cellular cycle. One of these solid tumor drugs was CMF (cytoxan, methotrexate and fluorouracil), a standard therapy for treating breast cancer for over 30 years. Understanding of molecular changes in cancer cells quickly developed after the 1970s. As a consequence, numerous drugs with various mechanisms of action were introduced during the 1980s. Subsequent advances and developments led to liposomal therapy, which places drugs inside liposomes (vesicles made of lipid bilayers), decreasing some of the side effects of chemotherapy such as cardiotoxicity. Examples of liposomal drugs include liposomal doxorubicin and daunorubicin, one of the first steps in nanotechnology-based approaches. The 1990s sparked the beginning of targeted chemotherapy by screening for specific critical molecular targets. These advances in modern chemotherapy and studies on genetics and molecular biology contributed to the ongoing decline in death rates. Data from the genome sequence suggested that many dysfunctions associated with cancer could be due to the abnormal function of some protein kinases. The current pharmacological trend has been to develop kinase inhibitors (Manning et al., 2005; Krause, 2005). The first tumors targeted with drugs approved by the FDA (Food and Drug Administration) and the EMEA (European Medicines Agency) were renal cell cancer, hepatocellular cancer and gastrointestinal stromal tumors. In recent years, numerous specific tumors have been tested with various kinase inhibitors and there is a trend towards combining chemotherapy with these new targeted therapies. Chemotherapy is curative in some types of advanced cancer, including acute lymphoblastic and acute myelogenous leukemia, Hodgkin's and non–Hodgkin's lymphoma, germ cell cancer, small cell lung cancer, ovarian cancer and choriocarcinoma. In pediatric patients, curable cancers include acute leukemia, Burkitt's lymphoma, Wilms' tumor and embryonal rhabdomyosarcoma. Although treatment is not always curative for these cancers, there has been significant improvement in progression-free and overall survival. Another modality of treatment is neoadjuvant therapy, which aims to reduce the size of the primary tumor and prevent micrometastases. This type of treatment improves on more conservative surgical techniques in preserving the functionality of important organs. Neoadjuvant chemotherapy is indicated for anal, breast, lung, gastroesophageal, rectal, bladder and head and neck cancer, as well as some types of sarcoma. There are many cancers for which adjuvant chemotherapy has been established with curative effect, and with the new effective drugs and combinations the curability rates are expected to rise even more. Since 1990, the incidence and mortality of cancer have been declining and despite the increase in the elderly population (De Vita, 2008), mortality rates for the United States declined from 2005 to 2007. In 1890, Halsted performed the first radical mastectomy, believing that cancer would be more curable if surgical techniques were more aggressive, thus avoiding regional recurrences. He had many followers at that time, but thanks to advances in chemotherapy, radiotherapy, biology and technology, the outlook now is quite different.

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Advanced cancer therapies

Radical surgery has now been replaced by less extensive operations. The turn of the 20th century marked the beginning of the development of cancer surgery techniques, with the first abdominoperineal resection performed in 1908 by Miles, 1908, the first Cancers 2011, 3 3282 lobectomy being performed in 1912 (Davies, 1914; Naef et al., 1993) and the first radical hysterectomy performed by Wertheim in 1906, all carried out under oncological criteria. Additionally, in 1904, Young made the first radical suprapubic prostatectomy. Modern surgery has changed significantly, with Halstedian techniques replaced by non-invasive procedures such as laparoscopic colectomy (for the removal of colon cancer) (Phillips et al., 1992), videothoracoscopy, radiofrequency ablation and radiosurgery techniques such as Cyberknife® (Sherwood et al., 2007). Breast-conserving surgery with sentinel-node removal has been used to improve esthetic results and avoid lymphedema (Singletary, 2001). Another example of conservative surgery is the use of laryngoscopic laser surgery in early laryngeal cancer (Genden et al., 2007). The most recent development is the Da Vinci®, a robotic system for the removal of cancer from prostate and kidney (Hashizume, 2007). The discovery of X-rays and radiation by Becquerel and Rontgen in the late 19th century was the first step towards radiation treatment. Marie Curie's work greatly contributed to the development of radiotherapy. The first cancer case cured exclusively by radiation occurred in 1898. After World War II, technological progress allowed charged particles to be propelled through a vacuum tunnel called linac, or linear accelerator. In 1960, Ginzton and Kaplan began to use a rotational linac radiotherapy called "Clinac 6", which was used to concentrate X-rays more deeply thereby they not affecting the skin as much. The development of modern computers enabled three-dimensional X-ray therapy, such as intensity-modulated radiation therapy (IMRT) using mapping information from Computed Tomography (CT) scans. This provides a three-dimensional reconstruction, which helps avoid toxicity since the contours of the tumor are targeted and separated from healthy tissues. In 2003, a specific type of IMRT was developed called the TomoTherapy® system. This treatment uses CT-guided IMRT technology that directs the radiation source by rotating it around the patient, which makes the morphological limits of a tumor easier to trace with the beam (Hall, 2006). Another significant trend is the use of charged particle radiotherapy with proton or helium ions for specific types of patients with melanoma of the uveal tract. It is also used as adjuvant therapy for skull base chondroma, chondrosarcoma and spine (usually cervical). In summary, the lines of development have been fractionated dose delivery, technological advances in X-ray production and delivery and improvement of computer-based treatment planning. The latest advance in scanning technology with radiotherapy therapy is four-dimensional (4D) conformal radiotherapy (Murphy, 2010), which records a video sequence of tumor movement. This therapy uses dynamic CT images of the body that compensate for any movement by the target, including movements when patients breathe. There are two forms of this therapy: Image-guided radiation therapy (IGRT) and Image-guided adaptive radiation therapy (IGART). Another combined system is radiogenic therapy, which induces the formation of cytotoxic agents against cancer cells. Lower doses of radiation are used along with a biological agent, and stimulation by radiation produces cytotoxic agents. This complex technology was developed to use radiation to activate

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promoters and thus inducing the expression of genes responsible for producing enzymes. These proteins activate the selected drug, and the activated form of the drug then destroys cancer cells. Another modality consists of radiolabeled molecules, which fight cancer by delivering targeted radiation to specific receptor-bearing cells. Radioactive isotopes (Iodine-125 or Indium-111) emit Auger electrons, which have the potential to be delivered to specific sets of target cells, thus sparing healthy cells.

Conventional Treatment for Thrombocytopenia

Platelet transfusion is often the only readily available treatment. With the discovery of 1thrombopoietin in 1994, great expectations were generated that it would play a role in preventing or treating thrombocytopenia in cancer patients, just as erythropoietin and granulocyte colony-stimulating factor (G-CSF) have played roles in reducing anemia and neutropenia, respectively (Kuter et al., 2014) The first-generation recombinant thrombopoietins reduced chemotherapy-related thrombocytopenia in early clinical trials, but their subsequent development was halted due to antibody formation against endogenous thrombopoietin (Kuter et al., 2002) While two second-generation thrombopoietin receptor agonists have now been developed that are potent stimulators of platelet production, neither has yet been tailored for treating thrombocytopenia in patients with cancer (Kuter, 2014: Kuter, 2013)

Therapeutic and prophylactic platelet transfusions create the additional risk of infusion complications. Thrombocytopenia can also occur with any infection or adverse drug reaction associated with cancer treatment.

Diagnosis of thrombocytopenia exacerbates the patient's sense of anxiety and fear beyond that associated with the cancer diagnosis itself. Clinicians' responses to thrombocytopenia in a cancer patient vary. Reduction of the dose intensity of chemotherapy or radiation is common; more effective regimens with thrombocytopenic toxicity may be avoided; and treatment may even be precluded. For some patients, treatment of the underlying cause of thrombocytopenia (eg, stopping therapy with the offending antiviral drug) may work.

Finally, the combination of natural bioactive compounds with traditional chemotherapeutic drugs can potentiate anti-cancer efficacy and reduce side-effects of chemotherapy. In some cases, addition of bioactive compounds may overcome the chemoradio-resistance of cancer cells. These synergistic effects of nutraceutical compounds such as flavonoids, stilbenes, terpenes, curcumin, and others have been reported and discussed in many reports and review article.

Herbal drugs (Phytochemicals) and thrombocytopenia

Management of thrombocytopenia currently includes pharmacological and surgical options. Platelet transfusion is preferred in case of emergency. However, the inability to provide the supportive treatment to increase platelet count often proves fatal to patients. Hence, the potentiality of antioxidants and various plant extracts, as substitutes to increase the platelet count are being investigated. Numerous plants are currently being used to boost platelet counts in thrombocytopenic disorders, including dengue hemorrhagic fever.

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Significance of the study

Cisplatin induced thrombocytopenia As platinum-based drugs always cause DNA damage in cancer cells, we can hypothesized that carboplatin inhibits bone marrow cell viability through inducing DNA damage. Therefore, expression of pH2A.X (S139), a DNA damage and mitotic marker in bone marrow cells treated with carboplatin. Western blot analysis showed that carboplatin dramatically increased the expression of pH2A.X indicating DNA damage induced by carboplatin in bone marrow cells. So platin based drugs can induce DNA damage in bone marrow cells to improve carboplatin-induced myelosuppression (Chen et al., 2017).

Improvement in platin-induced DNA damage by activating the DNA repair pathway by a combination of nucleotide excision repair (NER) and homologous recombination can be the best therapy in bone marrow cells to augment thrombocytopenia.

If the similar phenomena happen in cancer cells, drastic cell multiplication along with antagonistic action against chemotherapeutic drugs can be happen. Which can worse the cancer condition?

The reports available till to date was reported about the efficacy of papaya leaf on thrombocytopenia in different disease conditions but no report is available on its antagonistic action against plantin based drugs and their proliferation/inhibitory action on cancer cells during chemotherapy.

MATERIALS AND METHODS

Chemicals and instruments

DMEM/F12, RPMI-1640 and MTT [3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide)] were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetic acid, dimethyl sulfoxide (DMSO) and trypsin were prepared from Merck (KGaA, Darmstadt, Germany).UV-Visible (UV-Vis) spectrometer(Analytik Jena AG, Germany), Incubator (JTSL 40; Jal Tajhiz, Tehran, Iran) and multi-mode reader (Synergy HTX; BioTek Instruments, VT, USA) were used.

Preparation of papaya leaf extract

Methanolic extract

The fresh leaves were washed, dried in the shade, and powdered. Then, 100 g of Baneh leaves were mixed with 500 mL of pure methanol and extracted at 80^0C temperature for 6hours. Later, the mixture was filtered. Repeat the same steps with 400ml and 300ml of pure methanol with the remaining spent in first step. After mixing the filtered solutions, concentrated on the rotary evaporator under reduced pressure. Finally, the crude extract was kept at -20°C for further investigations.

Alkoloids Estimation in *papaya extract* sample by Gravimetric Method as follows

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- Accurately weigh 5 g of the sample in 50 ml distilled water.
- Acidify with 2 ml conc. Hydrochloric acid and filter solution.
- Wash the solution with 25 ml Chloroform two times.
- Discard Chloroform layer.
- Bring the pH to 9-10 using Ammonia solution.
- Extract the solution with 25ml, 20 ml, 15 ml, 15 ml and 10 ml Chloroform.
- Collect the Chloroform layers.
- Wash the Chloroform layer with 25 ml water.
- Evaporate the Chloroform layer and dissolve the residue in 5 ml Methanol.
- Dry the Methanol and weigh the residue.

Calculations:

$$\% \text{ Alkaloids} = \frac{\text{Wt. of the residue}}{\text{Sample weight}} \times 100$$

Cell Lines: Human breast cancer cell line MCF-7 were used for current studies

Cell culture condition

The L929 and MCF-7 cell lines were cultured in the RPMI medium containing 10 mg/L streptomycin, 100 IU/mL penicillin, and 10% of fetal bovine serum (FBS) under sterile conditions in the 5% CO2 incubator at 37°C (Memmert, Germany). The culture medium was fed every 1-2 days with a fresh medium.

Sub-culturing cells

The medium was aspirated from cultured T25 flask, the cell monolayer was washed with 2 ml of Trypsin-EDTA solution by gently rocking the flask back and forth several times. The Trypsin-EDTA solution was aspirated and the cell monolayer was incubated with 4ml of fresh Trypsin-EDTA for 5-10 min at 37^0C. The flask was gently tapped to ensure the cells were detached into the solution and to this 6mL of fresh cell culturing medium was added.

Add $1*10^5$ cells into 10ml of media in T25 flask and incubate at 37^0C

Cell Viability using Trypan Blue

Procedure

Bring adherent and semi-adherent cells into suspension using trypsin/EDTA as described previously and resuspend in a volume of fresh medium at least equivalent to the volume of trypsin. For cells that grow in clumps centrifuge and resuspend in a small volume and gently pipette to break up clumps. Under sterile conditions remove 100-200ul of cell suspension. Add an equal volume of Trypan Blue (dilution factor -2) and mix by gentle pipetting. Clean the haemocytometer. Moisten the cover slip with water or exhaled breath. Fill both sides of the chamber with cell suspension (approximately 5-10ul) and view under an inverted microscope using x10 magnification. Count the number of viable (seen as bright

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cells) and non-viable cellsi stained blue). Ideally >100 cells should be counted in order to increase the accuracy of the cell count. Note the number of squares counted to obtain your count of >100.

Calculate the concentration of viable and non-viable cells and the percentage of viable cells using the equations

Percentage of Viability = (No. of Viable cell/ Total Number of cells)*100

Cell viability Assay using MTT

The cytotoxicity assay was carried out according to Mosmann (Mosmann, 1983) with moditications according to (Carmichael et al, 1987). The assay is based on the reduction of MTT (3. (4, 5-dimethylthiazol2-yl) 2, 5 diphenyltetrazoliurm bromide) by the mitochondrial dehydrogenase of viable cells to blue formazan product which can be read spectrophotometrically.

Cells were trypsinized to detach them from the surface and appropriate amount of medium was added to make a cell suspension, 10ul of the cell suspension is loaded on to a Neubauer chamber (#AC 1000, Hawksley, Marlborough Road, Lancing Business Par, Lancing, Sussex, BN15 8TN) and the total number of cells in the eight corner squares was counted under the micioscope. The average of the cells was found and multiplied with 10• to get the number of cells/ml.

10000 cells/200 ul of medium per well is calculated and plated in 96 well plate (Sarstedt Inc.Newton, NC 28658, USA). The cells were incubated for overnight at 37^0C allowing them to settle and the medium was aspirated with the help of sterile micropipettes connected to a vacuum pump The stock solutions of the extract were prepared in DMEM at the required concentration. Fresh medium containing different concentrations 2, 5, 10 and 15µg/ml of Curcumin as standard and 2.5, 5.0, 10, 25 and 50 µg/ml of papaya extract was added to the wells and incubated for 48hr at 37^0C. The medium was aspirated and 300ul of MTT (0.5 mg/ml of medium) was added to each well 3h before the completion of the time period. The plate was then incubated for 3h at 37^0C and 1000ul of DMSO was added to each well in the plate to soluble the insoluble formazan crystals and the plate was incubated at 37^0C for 15 min, The wells were read at 540 nm in a spectrophotometer. The 0.D are plotted on a graph.

Immunofluorescence:

Cells were cultured on glass coverslips in 12well plates (Sarstedt Ltd, 68 Boston Road, Beaumont Leys, Leicester, LE4 1AW, UK) to reach approximately 70% confluence in 12-well tissue culture plates. The cells were washed with PBS and fixed for 5 min with acetone/methanol (-20°C, 1:1 v/v). The cover slips were then placed onto a drop of Prolong" Gold antifade reagent with DAPI (#P36935, Invitrogen") mounting medium prior to

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immediate fluorescence microscopy. Cells were visualized using a fluorescence microscope Magnification 40X.

Synergetic and/ antagonistic effect of aqueous papaya extract comparative to Hydroalcholic extract:

Cells were trypsinized to detach them from the surface and appropriate amount of medium was added to make a cell suspension, different groups were maintained by addition of $7*10^5$ cells into each T25 flask. Treatment was done as per below table, by addition of 10ml culture medium dissolved with required concentrations of drug & extracts and incubated at 37^0C for 48hr..

The concentration at which Hydroalcohalic extract exerted maximum proliferative efficacy on stem cells was selected for current studies (data unpublished)

1. Untreated
2. Cisplatin (100µM/ml)
3. Cisplatin (100µM/ml) + Compounds enriched ext (2.0µg/ml)
4. Compounds enriched ext (2.0µg/ml)

After the treatment period, trypsinization was done. cell count and cell viability (by tryptophan blue dye) was done by using haemocytometer as described before.

Gene expression studies: DNA isolation:

Take MCF-7 cells and remove the media. Add ice cold PBS and centrifuge at 3000 rpm for 10 minutes at 4 ^{o}C., Resuspend the cells in ice cold PBS and centrifuge at 5000 rpm for 5 minutes. Remove the supernatant and pellet to add 1 ml of lysis buffer without RNAase. Incubate at 95^0c for 10 minutes[Dry bath]. Add 50 microliters of protenase-K .Incubate at 55^0c for 30 minutes.Than heat the solution at 95^0c for 10 minutes. And add 7 microliters of RNAase. Incubation for 1 hour at 37^0c .Than take equal volumes of phenol buffer is added[P^H-8] than incubate room temperature for 10-60 minutes. Centrifugation at 10000 rpm for 15 minutes at room temperature. To aqueous to add 0.2 volumes of ammonium acetate , 2 volumes of ethanol. Than centrifuge at 10000 rpm for 5 mins at room temperature. Wash with 70% ethanol and centrifuge at 6500 rpm for 5 minutes. Add TE buffer to the precipitate DNA and dissolve the TE buffer. Than run the agarose gel electrophoresis.

Buffers and solutions : Agarosesolutions, Ethidiumbromide, Electrophoresis buffer.

Nucleic Acids and Oligonucleotides: DNA samples, DNA Ladders.

(Samples of DNAs of known size are typically generated by restriction enzyme digestion of a plasmid or bacteriophage DNA of known sequence).

Prepare a 50x stock solution of TAE buffer in 1000ml of distilled H_2O:

For this weigh 242 g of Tris base in a chemical balance. Transfer this to a 1000ml beaker.

Prepare EDTA solution (pH 8.0, 0.5M) by weighing 9.31g of EDTA and dissolve it in 40ml distilled water. EDTA is insoluble and it can be made soluble by adding sodium hydroxide

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pellets. Check the pH using pH meter. Make the solution 100ml by adding distilled water. Pipette out 57.1 ml of glacial acetic acid. Mix the Tris base, EDTA solution and glacial acetic acid and add distilled water to make the volume to 1000ml.

Prepare sufficient electrophoresis buffer (usually 1x TAE) to fill the electrophoresis tank and to cast the gel:

For this we take 2ml of TAE stock solution in an Erlenmeyer flask and make the volume to 100ml by adding 98ml of distilled water. The 1x working solution is 40 mM Tris-acetate/1 mM EDTA. It is important to use the same batch of electrophoresis buffer in both the electrophoresis tank and the gel preparation.

Table-1: Prepare a solution of agarose in electrophoresis buffer at an appropriate concentration:

For this current study usually 2 grams of agarose is added to 100ml of electrophoresis buffer.

Agarose Concentration in Gel (% [w/v])	Range of Separation of Linear DNA Molecules (kb)
0.3	5-60
0.6	1-20
0.7	0.8-10
0.9	0.5-7
1.2	0.4-6
1.5	0-2-3
2.0	0.1-2

Preparation:

Allow the gel to set completely (30-45 minutes at room temperature), then pour a small amount of electrophoresis buffer on the top of the gel, and carefully remove the comb. Pour off the electrophoresis buffer. Mount the gel in the electrophoresis tank. Add just enough electrophoresis buffers to cover the gel to a depth of approx. 1mm. Mix the samples of DNA with 0.20 volumes of the desired 6x gel-loading buffer. Slowly load the sample mixture into the slots of the submerged gel using a disposable micropipette or an automatic micropipettor or a drawn-out Pasteur pipette or a glass capillary tube. Load size standards into slots on both the right and left sides of the gel. Close the lid of the gel tank and attach the electrical leads so that the DNA will migrate toward the positive anode (red lead). Apply a voltage of 1-5 V/cm (measured as the distance between the positive and negative electrodes). If the electrodes are 10cm apart then run the gel at 50V. It is fine to run the gel slower than this but do not run it any faster. Above 5V/cm the agarose may heat up and begin to melt with disastrous effects on your gel's resolution. If the leads have been attached correctly, bubbles should be generated at the anode and cathode.

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Run the gel until the bromophenol blue and xylenecyanol FF have migrated an appropriate distance through the gel. **(The presence of ethidium bromide allows the gel to be examined by UV illumination at any stage during electrophoresis).**

The gel tray may be removed and placed directly on a trans illuminator. When the UV is switched on we can see orange bands of DNA.

RNA isolation

Remove medium rinse the cells in 5-10 ml of ice cold PBS and resuspend the cells in 5-10 ml of ice cold PBS. Remove PBS and lyase the cells in 1 ml of solution-D per T25-flask. The cell suspension is transferred to a new tube. Homogenize the lysates at room temperature for 15-30 secs. Transfer the homogenate to a fresh tube and sequentially add 0.1 ml of 2 microliters sodium acetate [PH -4] 1 ml of phenol and 0.2 ml of chloroform-isoamyl alcohol per milliliter[ml] of solution-D .after addition of each reagent ,cap the tube and mix the contents thoroughly by inversion [49:1 V/V]. Vertex the homogenate vigorously for 10 secs . Incubate the tube for 15 minutes on ice to permit complete dissociation of nucleo protein complexs. Centrifuge the tube at 10000 rpm [12000rpm] for 20 minutes at 40 c and then transfer the upper aqueous phase [0.6 ml] containing the extracted RNA to a fresh tube .Do not take the bottom aqueous phase layer.

- Add an equal volume of isopropanol to the extracted RNA .Mix the RNA to precipitate for 1 hour (or) more at -200 c. Collect the precipitated RNA by centrifugation at 10000 rpm for 30 minutes[60 mins] at 40 c. Carefully decant the isopropanol and dissolve the RNA pellet in 0.3 ml of solution-D for every 1ml of this solution used in step-1. Transfer the solution to a microfuge tube vertex it well and precipitated the RNA with 1 volume of isopropanol for 1 hour (or) more at -200 c. Collect the precipitated RNA by centrifugation at maximum speed per 10 minutes at 40 c in a microfuge wash the pellet twice with 75% ethanol centrifuge again and remove any remaining ethanol with a disposable pipette tip . store the open tube on ethanol to evaporate.

- Add 50-100 ml of DEPC –treated H20 store the RNA solution at -700 c.

Solution –D:

- 4 M Guanidinium thiocyanate.
- 25 MM sodium citrate .
- 0.5% (w/v) sodium lauryl sarcosinate.
- 0.1 M beta- mercaptho ethanol. Dissolve 250 grams of guanidinium thiocyanate in 293 ml of H20, 176 ml of 0.75 M sodium citrate (PH -7.0) and 26.4 ml of 10% (w/v) sodium lauryl sarcosinate .Add magnetic bar and stirr the solution on a combination . Heater –stirrer at 650 c until all ingredients are dissolve store solution-D at room temperature and Add 0.36 ml of 14.4 M stock beta- mercapto ethanol per 50 ml of solution-D just before use solution-D (Store at room temperature).

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Stocks:

1. 250 grams of guanidinium thiocyanate 293 ml of water . 17.6 ml of 0.75 M sodium citrate(PH - 7.0). 26.4 ml of 10% sarcosinate at 650 c.

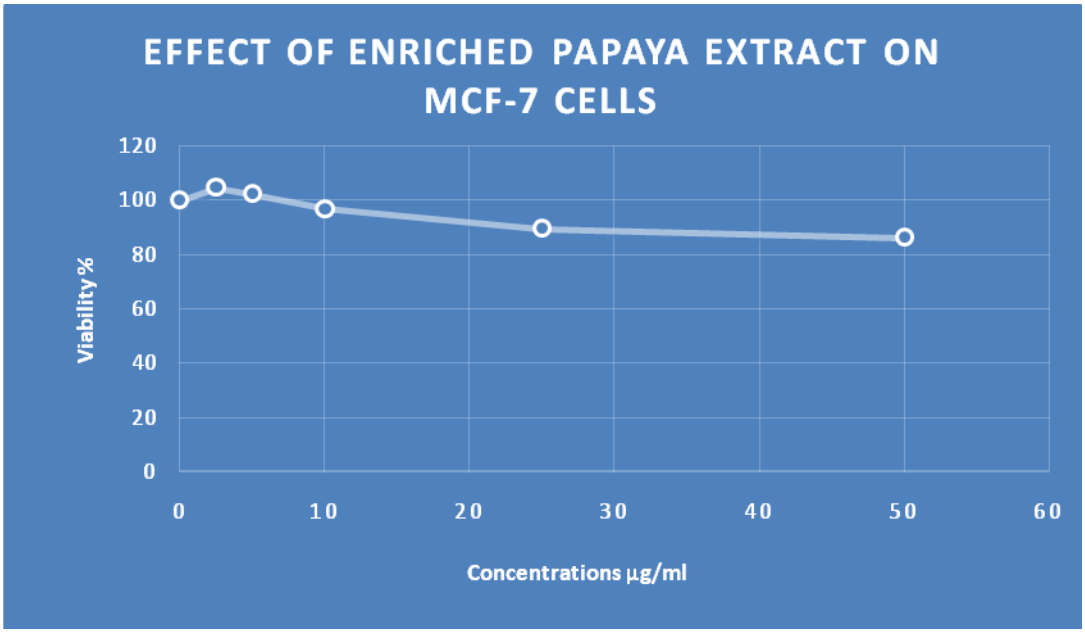
2. 5.0 gm / 6 ml guanidinium thiocyanate and add lysis buffer and incubate at 370 c for one and half an hour.

RESULTS:

Cell Viability using Trypan Blue

$75X10^4$cells/ml was seeded into each T25 flask. Confluence of the cells were checked by using inverted microscope after incubation at 37^0c at time interval of 24 hours and 48 hours . 10 microlitres of medium with cells after trypsinisation was loaded on to haemocytometer and cell count was done by electron microscope.the cell count after 48 hours incubation was $250X10^4$cells/ml.

Cytotoxicity by MTT assay



In the present study, 96 well plate was seeded with $1X10^4$cells/ml (200µl/well) and incubated overnight to make cells adsorbed to the bottom of wells. Then medium was carefully removed and added with 200µl of medium with different concentrations (2.5, 5.0, 10, 25 and 50µl/ml) of hydoalcohalic extract extract was added in triplicate and incubated for 48hr. 200µl of MTT (5mg/ml) was added and plate was incubated at 37^0C for 6hr in 5% CO_2 incubator. Settled Farmazone crystals at the bottom were observed. Carefully upper medium was removed. The crystals were dissolved in 500µl of DMSO in each well by micropipette.

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Mix the content well by using micropipette and left for 45sec. presence of viable cells was visualized by the development of purple color.

After complete dissolved crystals, the 96well plate was added by using microplate reader at 595nm and 630nm against DMSO as blank.

Standard graph was plotted by taking concentration of the extract on X-axis and cell viability percentage on Y-axis

Cell viability percentage calculation:

$$\% \text{ cell viability} = \frac{\text{Mean } OD_{595} - \text{Mean } OD_{630}}{\text{Control OD}} \times 100\%$$

In the present study, among the different concentrations, 50µg/ml of hydroalcohalic extract with 5% alkaloids showed maximum cell inhibitory activity (13.60%).

Confluence of cells during the treatment period (10X):

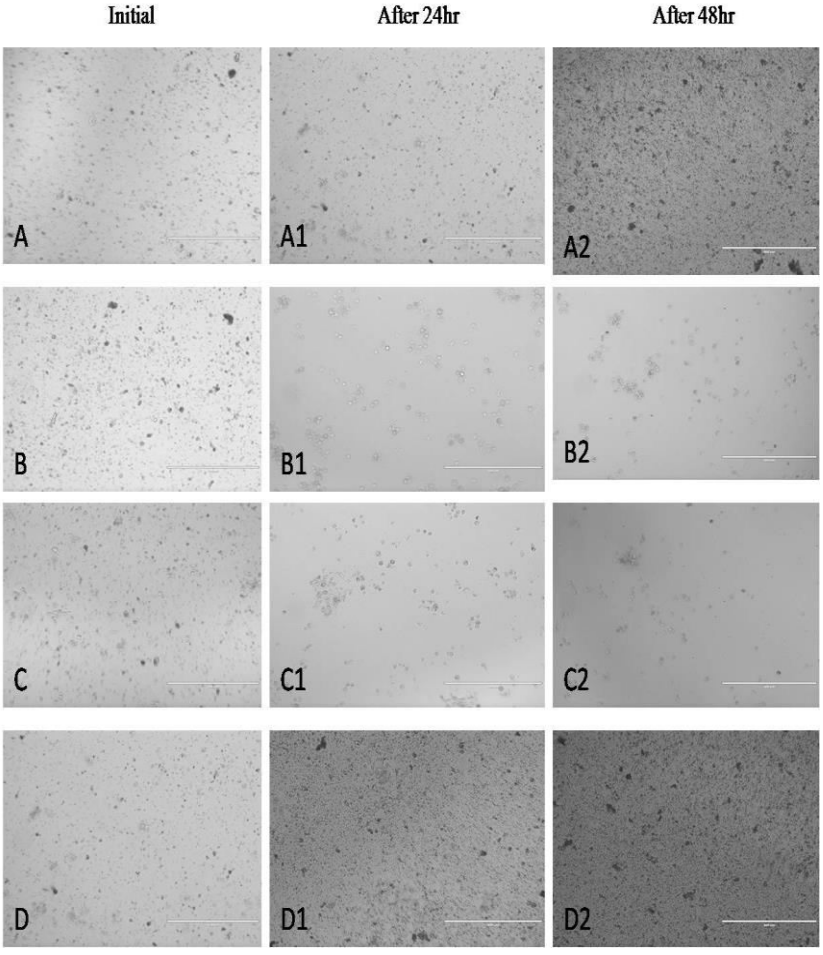

Figure-2: From the above results it is clear that after 24hr of incubation period, the confluence is 60% and after 48hr the confluence is almost above 90%. So further studies to test drug efficacy were planned for 48hr.

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4.4.Immunofluorescence (images):

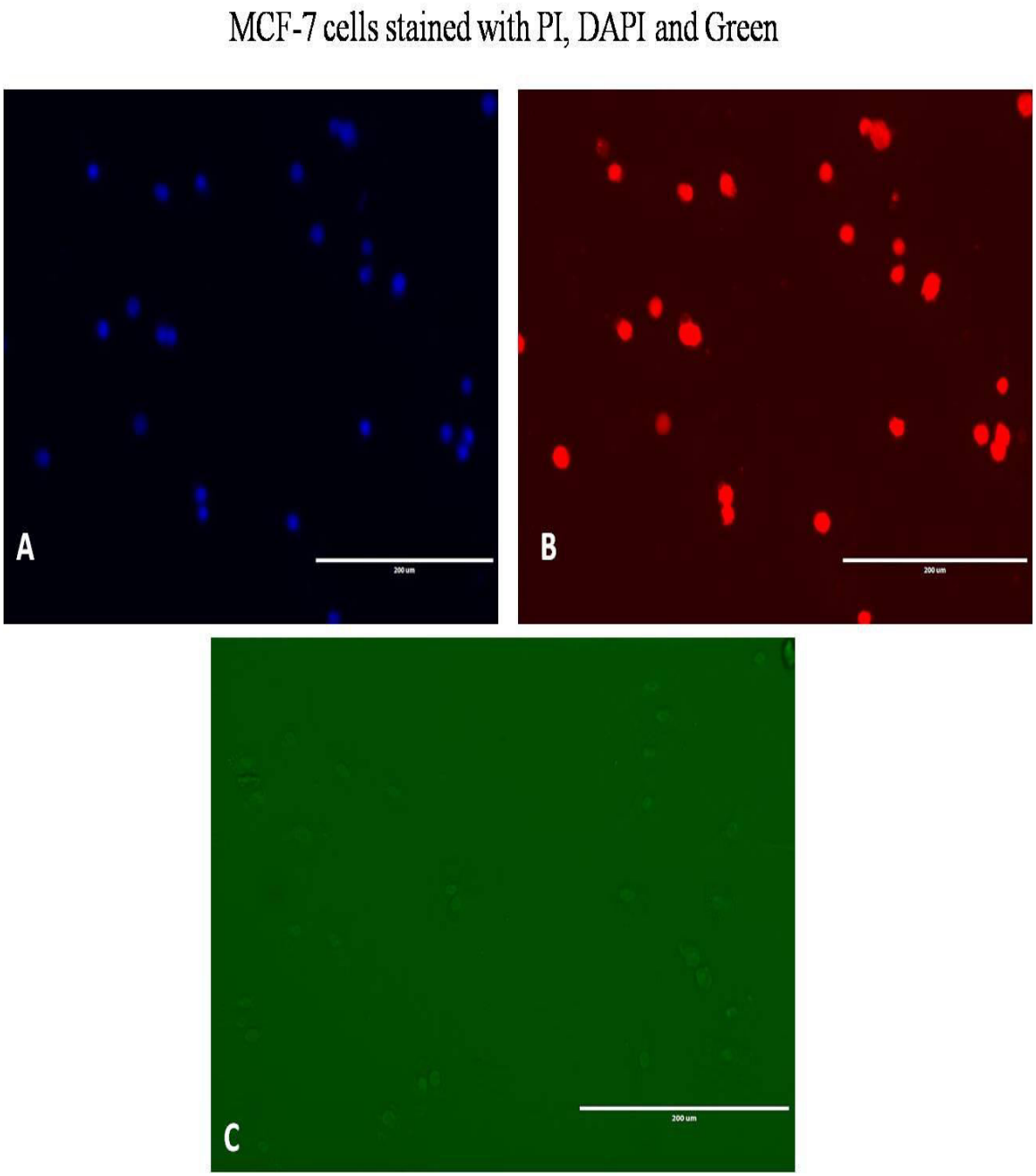

Figure-3: Trypsinized cells from subculture T25 flask were taken and seeded in 12 well plate with cover slips placed in each well and added with 500µl of medium into each well. Plate was incubated at 37^0C overnight to settle the cells on cover slip.

After incubation, coverslips were carefully removed and washed with PBS buffer. Then stain with DAPI, PI and green fluorescence (1µg/ml) and incubated in a dark for 5min. to remove excess stain, cover slips were washed with PBS.

The present results clearly showed the stained nucleus under inverted fluorescence microscope.

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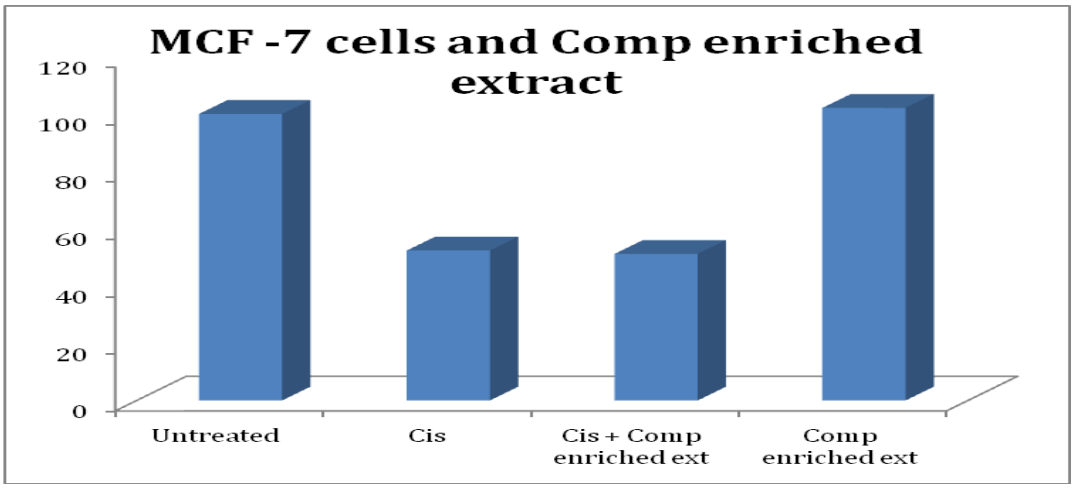
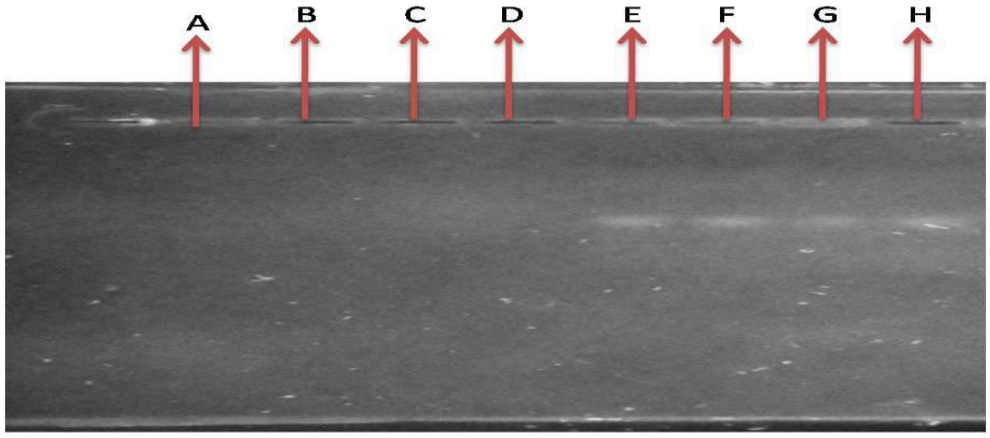
Graph-1: Drug interaction studies between cisplatin and Comp enriched extract of papaya (antagonistic effect studies)



The present study results study results clearly indicates comp enriched papaya extract (2µg/ml) showed no inhibitory effect on the MCF-7 cells when compared with the untreated cells. So the aqueous extract do not possesses cell inhibitory effect on the MCF-7 cells.
Whereas cisplatin treated MCF-7 cells showed much significance on cell count enhancement and cell viability percentage (52.34% decrease) when compared with untreated cells. The present results indicate that cisplatin can inhibit MCF-7 cells successfully by 50% at the concentration of 100µg/ml.

In MCF-7 cells treated with both cisplatin and comp enriched papaya extract, the cell count and cell viability percentage (51.17% decrease) is more or less equal and both the parameters are significantly lower when compared with untreated cells. The present results indicate that cisplatin has good inhibition on efficacy on MCF-7 cells.

Figure.3. Agarose gel electrophoresis: Qualitative analysis of BAD gene expression after subjecting to PCR.



A – Untreated
B – Aqueous extract
C — Hydroalcohalic extract
D — Compounds enriched ext
E — Cisplatin
F – Cisplatin + Aqueous extract
G – Cisplatin + Hydroalcohalic extract
H– Cisplatin + Compounds enriched ext

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DISCUSSION:

Thrombocytopenia is a condition where the platelet count is below 100000/µL. This condition arises from bone marrow suppression. Bone marrow suppression is a major pathological problem in human diseases like dengue (Kuter, 2013). Apart from viral infections, even drugs used to improve the health condition in some pathological conditions can induce myeloid suppression as side effect. One of the major drugs that can induce bone marrow suppression is chemotherapy drugs. There are different types of chemo agents that can suppression stem cells proliferation and differentiation, such that the health complications like decrease total CBC count. There are many drugs to treat decrease RBC, WBC and other blood cells production. Platelets production enhancement therapy is the most existing problem and other blood cells can be improved with the available treatments (Bonadonna et al., 1995; Chang, 2000).

Thrombocytopenia is a condition where the platelet count is below 100000/µL. platelet transfusion is the only option which has its own limitations in medical management. Papaya leaf extract is well established herbal medicine in the management of thrombocytopenia. In conventional treatments available to treat myeloid suppression, there is no single drug to take care of all the complications in such medical emergency. According to Hussan *et al*., 2017, papaya extract can inhibit bone marrow suppression caused by different chemo agents that acts at different levels of cell lineages in hierarchy of megakaryopoiesis and thrombopoiesis i.e cell lineages like haematopoietic stem cells, CLP, CMP ETC.

In the present study, among the different concentrations, 50µg/ml of hydroalcohalic extract with 5% alkaloids showed maximum MCF-7 cell inhibition (13.6%). For further studies we have selected 20µg/ml of aqueous extract as it showed maximum efficacy on stem cell proliferation.

In our basic study, we have taken mesenchymatous stem cells that can proliferate and differentiate into different cell lineages to maintain total CBC count at normal physiological conditions. Hydroalcohalic extract (5µg/ml) with 5% alkaloids, aqueous extract (20µg/ml) with 1% alkaloids and compound enriched extract (2µg/ml) with 20% alkaloids showed maximum cell proliferation and cell viability. Alkaloids are the pharmacologically active compounds that can acts against the myloid suppression (data unpublished).

In previous studies it is proved that papaya extract acts on megakaryocytes and increased the platelet production (REF). With the study we can prove that papaya extract can act on pluripotent stem cells i.e on parental cell for the reported megakaryocytes of previous studies and can contribute for their proliferation.

Cisplatin can induce apoptosis by inducing alkylation in the DNA strand and drives the cells towards the death (REF). Cisplatin was reported to induce cytotoxicity in megakaryocytes in similar way by promoting the proapototic genes and causes cell death (REF). From the studies of Hussan *et al*., 2017, papaya can inhibit cisplatin induced thrombocytopenia, indicates that papaya extract might suppress the proapoptotic gene expression.

If proapoptotic gene was suppressed by papaya extract in cancer cells then papaya extract cannot be used in the management of thrombocytopenia in chemotherapy undergoing cancer patients.

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In the present study, comp enriched papaya extract did not showed either inhibition or proliferation efficacy on MCF-7 cells when compare with untreated cells. But cisplatin could be able to inhibit MCF-7 cell by 50% at 100µM/ml. when MCF-7 cells treated in combination of comp enriched papaya extract and cisplatin, the inhibitory effect is more or less equal to MCF-7 cells treated with cisplatin. Results indicate that papaya extract could not induce proliferation capacity and could not affect efficacy of cisplatin on cancer cells.
MCF-7 cells were stained with DAPI, PI and green Fluorescence resulted in clear demarked nucleus.

BAD genes are proapoptotic genes that can activate the process of apoptotic cell death. cisplatin can induce cell death by activation of apoptosis pathway (Chen et al., 2017). It is well established fact that MCF-7 cells possess reduced apoptotic gene expression. In qualitative analysis of BAD gene expression studies by PCR and agarose gel electrophoresis, no clear band was observed in the untreated cells, comp enriched papaya extract treated and could able to observe the bands in the cells treated with cisplatin and comp enriched papaya extract + cisplatin. With the results it is clear that cisplatin induced apoptosis was not antagonised by papaya extract. Finally, It was concluded that papaya extract can enrich synergetic and antagonistic effect against cisplatins included programmed cell death in MCF-7 cell line.

Conflict of interest: NIL

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