COMPARATIVE CHEMOPREVENTION EFFECT BETWEEN *MANGIFERA INDICA* AND *PELTHOPHORUM PTEROCARPUM* ON COLON CANCER CELL LINE

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by

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LIST OF ABBREVIATIONS

µl	Microliter
AIF	Apoptosis Inducing Factor
APC	Adenomatous Polyposis Coli
APS	Ammonium PerSulphate
BB	Binding Buffer
BSA	Bovine Serum Albumin
CARIF	Cancer Research Initiative Foundation
CDK	Cyclin Dependent Kinases
CKI	Cyclin Dependent Kinases Inhibitor
CO_2	Carbon dioxide
DD	Death Domain
ddH_2O	Double-distilled water
DED	Death Effector Domains
DISC	Death Inducing Signaling Complex
DMEM	Dulbecco's Modified Eagles Medium
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
ECL	Enhanced Chemiluminescene
EDTA	Etylenediaminetera Acetic acid
EEMP	Ethanolic Extract of Mango Peel
ERK	Extracellular-Signal Regulated Kinases
FADD	Fas-Associated Death Domain

FAP	Familial Adenomatous Polyposis
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FITC	Fluorescein Isothiocyanate
GSK-3β	Glycogen Synthase Kinase 3-Beta
HNPCC	Hereditary Non-Polyposis Colorectal Cancer
HT-29	Human Colon Adenocarcinoma Cell Line
IBD	Inflammatory Bowel Disease
IC	Inhibitory Concentration
INK4	Inhibitors of CDK4
kDa	Kilo dalton
MAPK	Mitogen Activated Protein Kinase
MI	*Mangiferaindica*
mg/ml	Miligram per milliliter
ml	Mililiter
NF-kβ	Nuclear Factor kappa B Cells
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
PAGE	Polyacrylamide Gel Electrophoresis
PBS	Phosphate Buffer Saline
PCD	Programmed Cell Death
PCNA	Proliferating Cell Nuclear Antigen
PI	Propidium Iodide
PP	*Pelthophorumpterocarpum*
PT	Permeability Transition
PTEN	Phosphatase and Tensin homolog deleted from chromosome Ten

rpm	Revolutions per minute
RIPA	Radio Immuno Precipitation Assay
ROS	Reactive Oxygen Species
SDS	Sodium Dodecyl Sulphate
TBE	Trypan Blue Exclusion
TBS	Tris Buffer Saline
TEMED	Tetramethylethylenediamine
TGS	Tris Glycine SDS
TNF	Tumor Necrosis Factor
TNFR	Tumor Necrosis Factor Receptor

PERBANDINGAN DI ANTARA KESAN KEMOPENCEGAHAN *Mangifera indica* DAN *Pelthophorum pterocarpum* ATAS SEL KANSER KOLON

ABSTRAK

Kanser kolon telah dilaporkan sebagai kanser yang paling kerap dan menduduki tangga yang kedua di kalangan lelaki dan wanita dan kejadian ini semakin meningkat setiap tahun. Kanser ini boleh dirawat jika ia dikesan pada peringkat awal. Walaupun terdapat banyak rawatan yang canggih dan maju seperti kemoterapi, radioterapi dan pembedahan untuk merawat kanser kolon , tetapi terdapat sebilangan besar pesakit yangmengalami metastasis.Sejak akhir-akhir ini, ahli sains mendapati mendapati bidang kemopencegahan menjadi salah satu sasaran yang paling berfaedah dalam mengenal pasti ejen semula jadi dengan keberkesanan dibuktikan terhadap sasaran molekul yang ditakrifkan. Kajian ini telah dijalankan untuk menentukan kesan tumbuhan tempatan, Mangifera indica (MI) dan Pelthophorum pterocarpum (PP) ekstrak daun pada sel-sel kanser kolon. Kajian ini dijalankan dengan melakukan dengan mendapatkan Kepekatan Perencatan (IC_{50}) kedua-dua tumbuh-tumbuhan, yang menyebabkan PP menghalang 50% daripada pertumbuhan sel-sel kanser pada kepekatan yang lebih rendah (0.1mg/ml) daripada MI yang menunjukkan perencatan kepekatan yang lebih tinggi (0.25mg/ml). Rawatan ini juga menunjukkan beberapa perubahan morfologi, seperti gelendong atau bentuk kenaikan pada bahagian-bahagian sel. Analisis tambahan telah dilakukan dengan menguji proses kitaran sel dengan sitometri aliran yang mendedahkan rawatan MI menyebabkan perencatan pada fasa G2 / M dan fasa S manakala PP sel-sel dirawat tidak menyebabkan apa-apa perubahan penting dalam HT-

29 sel-sel.Keputusan analisis ‘Western blot’ mendedahkan bahawa kedua-dua MI dan ekstrak PP menunjukkan apoptosis melalui laluan ekstrinsik. Ini disokong oleh expresi protein Caspase 8 dalam sel-sel rawatan berbanding dengan kawalan. Hasilnya ada hubung kait dengan MI yang menyebabkan penurunan expresi Caspase-3 protein,. Data dipamerkan dalam kajian ini dapat disimpulkan bahawa kedua-dua MI dan PP ekstrak boleh digunakan sebagai agen chemoprevention kerana mereka memiliki sifat-sifat anti-kanser.

COMPARATIVE CHEMOPREVENTION EFFECT BETWEEN *Mangifera indica* AND *Pelthophorum pterocarpum*ON COLON CANCER CELL LINE

ABSTRACT

Colon cancer has been reported as the second most common cancer among men and women and the incidence is increasing every year. This cancer can be treated if it is detected at the early stage. Even though there are many advanced treatments such as chemotherapy, radiation to treat colon cancer, there are great numbers of patients who suffer from metastases. Recently, scientist have found interest in chemoprevention studies where it has become one of the most advantageous targets in identifying natural agents with demonstrable efficacy against defined molecular targets. Thus, this study was conducted to determine the effects of local plants, *Mangiferaindica*(MI) and *Pelthophorumpterocarpum*(PP) leaf extracts on colon cancer cells (HT-29). The study was conducted by obtaining the Inhibitory Concentrations (IC_{50}).PP inhibits 50% of the growth of cancer cells at lower concentration (0.1mg/ml) than MIwhichinhibited at higher concentration (0.25mg/ml). The treatment also showed some morphological changes, like spindle or spike shape of the cells.Additional analysis was done by testing the cell cycle deregulation with flow cytometrywhich revealed that MI treatmentinduced G2/M phase and S phase arrest while PP treated cells did not cause any significant changes in HT-29 cells. Western blot results revealed that both MI and PP extracts showed apoptosis through extrinsic pathway. This is supported by the down-regulation of Caspase 8 in treatment cells compared to untreated.

The result correlates with Caspase 3 protein, where MI inhibits the expression of Caspase 3. It can be concluded that both MI and PP extracts can be used as chemoprevention agents because they possess anti-cancer properties.

CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

Colon or rectal cancer, also known as collectively as cancer of the bowel, is the most common internal cancer among men and women (Jemal et al., 2010). Colorectal cancer is classified as one of serious health problems in most developed countries and is the third leading cause of cancer mortality throughout the world (Pisani et al., 1993). Piyush Gupta, states that in developing countries, infectious agents play a major role in cancer (stomach, liver, uterine cervix and esophagus), the attributable causes in developed countries are lifestyle and dietary factors (lung, colorectal, and prostate).

Changes in diet and lifestyle can reduce the risk of developing the third most prevalent form of cancer, which is colon cancer. Colon cancer incidence continues to rise among western populations due to their diet (Johnson *et al*., 2007). The authors conclude that adherence to these controllable lifestyle patterns can reduce risk of this deadly disease. '*You are what you eat* or *the foods you eat daily determine the diseases you will develop in later life` best describes on treating or preventing oneself from this disease*. Studies have proven that bioactive compounds such as phenolics, flavanoids, mangiferin, carotenoids, etc which can be identified in plants contains health maintaining properties (Vanamala *et al*., 2006). Intake on these fruits and vegetables prevents one from several ailments like cancers, diabetes, and osteoporosis.

They possess antioxidant and anticarcinogenic activities which help organisms deal with oxidative stress, caused by free radical damage, which cause the formation of Reactive Oxygen Species (ROS) that leads to many kinds of disease mainly cancer (Ali *et al*., 2008). Therefore, many medicinal plants and fruits have been the choice as an alternative medicine or treatment in treating or preventing cancer from occurring. This caused many researchers in Malaysia to study these plants that are found abundantly because of their great potential of chemopreventive action. This natural practice of treatment using plants is known as chemoprevention. Chemoprevention offers an attractive and mechanism-based approach to control the cancer development.

In this study, two local plants were used; *Mangifera indica* and *Pelthophorum pterocarpum*. Mango plant selectively from MI has been the focus for many scientists in searching this plant as the next potential antioxidant. Mango is a common garden tree all over the tropics. The fruit which is particularly high in Vitamin A is also, an important source of nutrition for birds, bats, insects and mammals. Its flowers serve as astringents to treat diarrhea, while the bark treats rheumatism. The leaf used as a remedy for fever, diabetes and hypertension. PP also known to be an ornamental tree, possess a wide range of medicinal properties. A research shows that this plant is traditionally used in the treatment of unhealthy skin, ringworm infection, constipation, insomnia, and stomatitis. In conventional medicine, PP flowers are used as an astringent to cure or relieve intestinal disorders after pain in childbirth, sprains, bruises and swelling or as a lotion for eye troubles, muscular pains and sores.

Thus, the focus of this study was to determine the effects of the crude extract of these two plants leaves on colon cancer cells by using cell based assays and to inhibit the

growth of the HT-29 cell lines. In addition, MI and PP will be compared to evaluate their effectiveness in inhibiting the growth of the cancer cell lines.

1.2 Hypothesis

The chemopreventive effects elicited by MI and PP natural dietary compounds of these plant extracts induce apoptosis, and modify cell cycle arrest.

1.3 Objectives

- To determine the inhibitory concentration dose (IC50) of MI, and PP on colon cancer cell line.
- To compare the anti-proliferative effect of MI, and PP on colon cancer cell line.
- To determine the apoptotic effect of MI, and PP on colon cancer cell line.
- To identify the modulation effect of MI, and PP on the cell cycle of colon cancer cell line.
- To determine the effect of MI and PP extracts on apoptosis marker expression such as Caspases and MAPK proteins.

CHAPTER 2

LITERATURE REVIEW

2.1 COLON

2.1.1 ANATOMY

Colon and rectum are situated at the last portion of the digestive system. They form a long, muscular tube known as the large intestine, where the first 4-5 feet of the large intestine is the colon and the end part is the rectum. The colon is made up of four sections; ascending colon, transverse colon, descending colon and sigmoid colon. Different parts of the colon are placed in two sides, which are intraperitoneal and retroperitoneal. Intraperitoneal organs, caecum, appendix, transverse coon and are completely enclosed by peritoneum and they are mobile. Retroperitoneal organs that is, the ascending colon, descending colon and rectum do not have a complete covering of peritoneum, so they are set in location.

The four sections of the colon:

- Ascending colon - It is the first part of the large intestine which is connected to the small intestine by the cecum. The ascending colon passes through the abdominal cavity, upwards toward the transverse colon almost eight inches (20 cm).
- Transverse colon - It is the part of the colon from the hepatic flexure to the splenic flexure (the turn of the colon by the spleen). The transverse colon hangs

off the stomach, attached by a wide band of tissue called the greater omentum. On the posterior side, the transverse colon is connected to the posterior abdominal wall by a mesentery known as the transverse mesocolon. The transverse colon is covered in peritoneum, thus it is movable.

- Descending colon – It is the part of the colon as of the splenic flexure to the beginning of the sigmoid colon. The function of the descending colon in the digestive system is to store faeces that will be emptied into the rectum.
- Sigmoid colon – It is the part of the large intestine after the descending colon and before the rectum. The name sigmoid means S-shape. The walls of the sigmoid colon are muscular, and contract to increase the pressure inside the colon, causing the stool to move into the rectum.

Colon is responsible for the last stages of the digestive process. The function of the colon is to remove water and nutrients from food and turns the rest into waste or better known as stool. This occurs after the digested food enters the colon from the small intestine. Stool or waste is passed to the rectum from the colon and out of the body through anus. It facilitates to sustain the body's fluid balance by absorbing valuable vitamins, and processes tough materials, such as fibers, and stores waste before it is eliminated.

In the colon, the mixture of fiber, small amounts of water, and vitamins, mix with mucus and the bacteria that live in the large intestine, begins the formation of feces. When the feces pass through the colon, the lining absorbs most of the water as well as some of the vitamins and minerals present. Bacteria inside the colon feed on the fiber,

breaking it down in order to produce nutrients that will nourish the cells that line the colon. Feces are moved along until the walls of the sigmoid colon contract, causing waste to move into the rectum through an action known as peristaltic movement. This wave-like motion encourages feces to move closer to the rectum and be expelled through the anus.

2.1.2 COLORECTAL CANCER

Colorectal cancer also identified as colon cancer, rectal cancer or bowel cancer is a cancer from wild cell growth in the colon or rectum, or in the appendix. Colon cancer is a cancer that grows in the intestinal gland cells that lines the inner part of the colon and rectum is an adenocarcinoma. It makes up 95% of colon and rectal cancers. Adenocarcinoma is a type of cancer that forms in mucus-secreting glands throughout the body. It can happen in many different places in the body. Genetic studies prove that essentially colon and rectal tumours are genetically the same cancer. Colorectal cancer begins in the innermost layer where it grows through some or all of the other layers.

A study that was conducted by Malaysia Cancer Statistics reported that ten leading cancers among population of Malaysia in 2007 were breast, colorectal, lung, nasopharynx, cervix, lymphoma, leukaemia, ovary, stomach and liver as can been seen in the Figure 2.1 and Table 2.1 (Zainal Ariffin Omar, 2011).

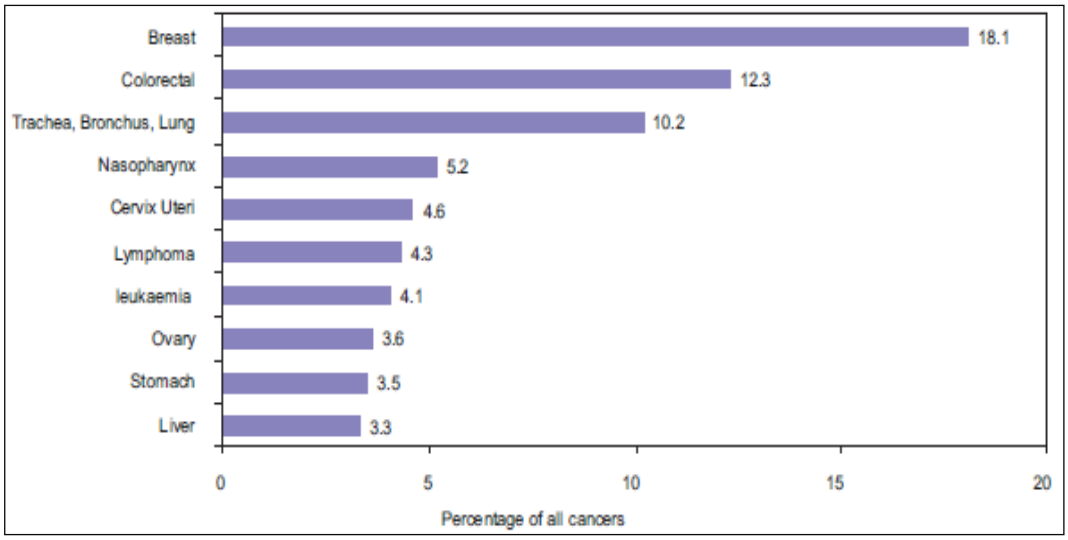

Figure 2.1: Ten most frequent cancers, all residence, Malaysia 2007

Table 2.1: Ten most frequent cancers, all residence, Malaysia 2007

ICD-10	SITES	NO	%
C50	Breast	3292	18.1
C18-C22	Colorectal	2246	12.3
C33-C34	Trachea, Bronchus, Lung	1865	10.2
C11	Nasopharynx	940	5.2
C53	Cervix Uteri	847	4.6
C81-C85,C96	Lymphoma	776	4.3
C91-C95	Leukaemia	741	4.1
C56	Ovary	656	3.6
C16	Stomach	630	3.5
C22	Liver	605	3.3

Table 2.2 and 2.3 summarize the five most frequent cancers among Malaysian males in 2007 as lung, colorectal, nasopharynx, prostate and lymphoma, while in the females were breast, colorectal, cervix, ovary and lung.

MALES					
ICD-10	SITES	NO	%	CR	ASR
C33-C34	Trachea, Bronchus, Lung	1320	16.3	10.3	14.8
C18-C20	Colorectal	1185	14.6	9.7	13.4
C11	Nasopharynx	685	8.4	5.4	6.4
C61	Prostate	502	6.2	3.9	6.2
C81-C85,C96	Lymphoma	448	5.5	3.5	4.2
C22	Liver	443	5.5	3.5	4.7
C91-C95	Leukaemia	419	5.2	3.3	3.5
C16	Stomach	351	4.3	2.7	3.9
C70-C72	Brain, Nervous system	259	3.2	2.0	2.3
C67	Bladder	258	3.2	2.0	2.9

Table 2.2: Ten most frequent cancers, males, Malaysia 2007

FEMALE					
ICD-10	SITES	NO	%	CR	ASR
C50	Breast	3242	32.1	26.0	29.1
C56 C18-C20	Colorectal	1011	10.0	8.1	10.2
C53	Cervix Uteri	847	8.4	6.8	7.8
C56	Ovary	656	6.5	5.3	5.9
C73 C33-C34	Trachea, Bronchus, Lung	545	5.4	4.4	5.6
C54	Corpus Uteri	414	4.1	3.3	3.9
C81-C85,C96	Lymphoma	328	3.2	2.6	3.0
C91-C95	Leukaemia	322	3.2	2.6	2.7
C73	Thyroid	305	3.0	2.4	2.7
C16	Stomach	279	2.8	2.2	2.8

Table 2.3: Ten most frequent cancers, females, Malaysia 2007

2.1.2 (a) Formation of Colon Cancer

Colon cancer development is often discussed at an early stage in the epithelium hyperproliferation where it leads to the formation of the adenomas. It is due to the dys-regulation of the cell cycle control. (Potten, 1997). Colorectal cancer process develops when there is disturbance in the replacement of normal lining cells which leads to the fault in mucosal cell division. This causes these cells to divide autonomously of the normal checks and balances that control growth. As these abnormal cells grow and divide, they can direct to growths within the colon known as polyps. Polyps vary in type, but many are precancerous tumors that grow slowly over the course of years and do not spread. As polyps grow, other genetic mutations become unstable and can make the cells stranger. Once these precancerous tumors change direction the precancerous polyps become cancerous. In most cases this process is slow, taking at least 8 to 10 years to develop. Figure 2.2 easily illustrates the point where the cancer begins and the polyp formation.

When a colorectal cancer is formed, it begins to grow in two ways. Firstly, the cancer grows locally and then extends through the wall of the intestine and attack adjacent structures, making huge mass. Second, as the cancer grows it begins the process of metastasis, shedding thousands of cells a day into the blood and lymphatic system that can cause cancers to form in distant locations. Colorectal cancers most commonly spread first to local lymph nodes before travelling to distant organs.

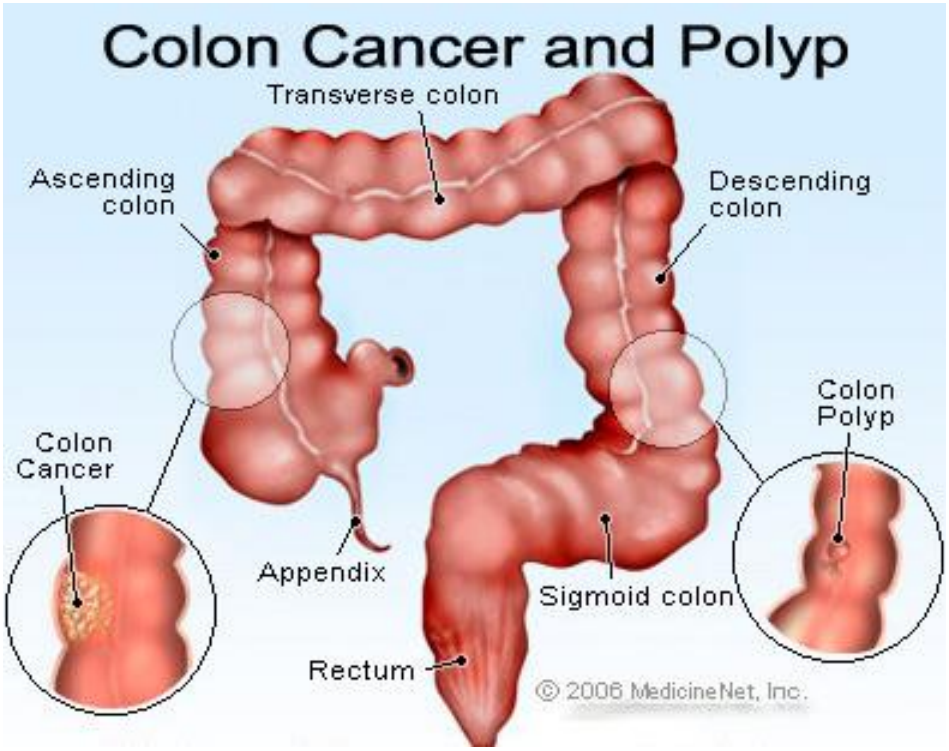

Figure 2.2: Picture of colon cancer (Adapted from Medicinenet.com)

2.1.2 (a) Risk Factor of Colorectal Cancer

There is no real cause for colon cancer or specific factors that lead to it. Colon cancer develops when the healthy cells in the colon are altered. Therefore, through many studies and researches have been conducted, there are few main risk factors classified as causes for colorectal cancer. They were divided into:

Dietary factors

The occurrence of colorectal cancer around the world varies depending on areas, such as highest incidence at North America, New Zealand, Europe and Australia compared to developing and not developed countries. Clinical research proposes that

difference in dietary patterns and economical growth plays role in the cancer incidence (Johnson *et al*., 2007). Lately, it was reported by the World Cancer Research and the American Institute for Cancer Research that inappropriate diet and lack of good physical activities contributes in causing colon cancer (Marmot, 2007). Research evidence shows that individuals who consume diet that is rich in fat, red meat and low in vitamins are prompted to colon cancer as stated by American Cancer Research. They also suggested that meats cooked at very high temperatures (frying, broiling, or grilling) causes chemicals to increase cancer risk. Therefore, intake of natural diet such as fruits and vegetables, or any natural food that contains high in vitamins and mineral has the choice in preventing or interrupting the process of carcinogenesis. This is because these natural diets were able to modify or delay process of carcinogenesis (Khan *et al*., 2006).

Non-dietary Factors

Smoking

A study that that was conducted by Lindsay *et al., (*2009), demonstrated that prolonged cigarette smoking leads to colorectal cancer. Smoking is also known as the precursor for cancer (Labianca *et al*., 2010). One in five colorectal cancer cases are caused by tobacco use in USA.

Inflammatory bowel diseases

Inflammatory bowel disease (IBD), which takes account of *ulcerative colitis* and *Crohn's disease*, is a state where the colon cells are inflamed for a long time. Prolonged IBD leads to dysplasia (cells in the lining of the colon or rectum looks different). These cells later on convert into cancer cells.

Precancerous growths

It is understood that colon cancer begins as clumps of precancerous cells or known as polyps. Polyps are one of the causes for colorectal cancer. Tumor or also known as growth of tissue starts as non-cancerous polyps on the inner lining of the colon, where later the polyps develop into either benign or malignant polyps. The chances of the polyp to be cancerous depend on the type of polyp; adenomatous polyps (adenomas) are polyps that can change into cancer. Hyperplastic polyps and inflammatory polyps are not pre-cancerous, but that certain hyperplastic polyps can become pre-cancerous or might be a indication of having a higher risk of developing adenomas and cancer, especially when they grow in the ascending colon.

Genetic Factors

Family health background also is an important factor in colon cancer risk. Close family members that have history of colorectal cancer are likely to develop this disease themselves, especially if the relative had the cancer at a young age. There are 2 groups of syndrome. The main syndrome is the familial adenomatous polyposis (FAP), which is associated with mutation or loss of FAP (also called the adenomatous polyposis coli—APC) gene (Labianca *et al*., 2010). Hereditary non-polyposis colorectal cancer (HNPCC) syndrome is the second most common type of inherited (genetic) colorectal cancer. It accounts for about 2 percent of all colorectal cancer cases. This is caused by changes in an HNPCC gene. In a book by NCI US, 2006, it states that most people with an altered HNPCC gene develop colon cancer, and the average age at diagnosis of colon cancer is 44 years old.

2.2 CANCER CHEMOPREVENTION

Cancer chemoprevention has caused great interest among clinicians and scientist in finding ways to prevent this killing disease. Cancer chemoprevention, as first defined by Sporn in 1976, uses natural, synthetic, or biologic chemical agents to reverse, suppress, or prevent carcinogenic progression (Sporn, 1976). It is based on the concepts of multifocal field carcinogenesis and multistep carcinogenesis. The victory of several recent clinical trials in preventing cancer in high-risk populations recommends that chemoprevention is a proper and appealing strategy. Significance in this area of research has tremendously increased with improved understanding of the biology of carcinogenesis and the identification of potential molecular targets to retard this process.

Food-derived products has become the main attraction for the development as chemopreventive agents because of their safety and the fact that they are not regarded as "medication," and they don't cause any side-effects from long-term use in populations at normal risk. Death causing cancers like, breast, prostate, colon and lung show promising results when treated with this numerous diet-derived agents and are being evaluated clinically as chemopreventive agents (Kelloff *et al*., 2000).

There are some examples of chemoprevention that are well known:

- Tamoxifen (Nolvadex), known as an estrogen blocker reduces the risk of developing breast cancer, and raloxifene (Evista), that lowers the risk of developing breast cancer in women who have been through menopause (Osborne et al, 1998).

- Finasteride (Propecia, Proscar) – A study demonstrates that bicalutamide and finasteride may be given as primary hormonal therapy for advanced prostate cancer with few side-effects (Tay *et al*., 2004)
- Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDS) lower the risk of many types of cancer in people with an average risk of cancer. Research based on animal model provided convincing evidence that administration of the NSAIDS inhibited chemically induced colon carcinogenesis(Agarwal *et al*., 2003)

The tradition of active medicinal compounds or extracts from traditional medicines or natural sources is well thought-out as one such substitute treatment approach. Various naturally derived compounds are known to be safe as they are obtained from frequently consumed food.Therefore,consumption of fruits and vegetables which contains antioxidants was believed to reduce the risk of any type of cancer, mainly colon cancer. *Cordyceps militaris* is a well-known medicinal mushroom that has been used in oriental medicine for treating various diseases, including cancers (Mohammad *et al*., 2012). A study done by Li *et al*., (2013) clearly suggest hawthorn species as a promising source for cancer prevention and treatment will certainly encourage future studies to increase our knowledge on the anticancer potential of this food plant.

2.3 *Mangifera indica* L

Mango or scientifically known as Mangifera indica (MI), has been an important plant in the Ayurvedic and native medical systems for over 4000 years. Mangoes belong to the flowering plant family Anacardiaceae and genus *Mangifera* which consists of about 30 species of tropical fruiting trees, as shown in Figure 2.3. MI is among the most economically and culturally important tropical fruits, especially in Asia. It was originally found in the foothills of the Himalayas in north-eastern India, Burma, and Bangladesh. It is now grown in most tropical countries and some subtropical countries. Mango was brought to Malaysia and other East Asian countries hundreds of years ago, then to East and West Africa.

MIhas been the focus of many scientists in searching this plant as the next potent antioxidant. Recently a study proves that the stem bark of this plant contains anti-inflammatory activity (Martinez *et al*., 2000). Other studies also showed that the extract from the stem bark of MI can reduce the production of ROS by peritoneal macrophages in mice (Sanchez *et al*., 2000). A study was conducted on leaves which reveals that the leaves contains high amounts of total phenolics and flavoids, which is known as an antioxidant (Elzaawely & Tawata, 2010). A study proves that the presence of phytoconstituents in the leaf extracts may be responsible for the antimicrobial activity. However, not much is known about the chemical composition of the plant leaves.

Figure 2.3: Picture of *Mangifera indica* plant

Mangifera indica L.

Kingdom: Plantae-Plants

Division: Magnoliophyta-Flowering plants

Class: Magnoliopsida-Dicotyledons

Order: Sapindales

Family: Anacardiaceae-Sumac family

Genus: Mangifera L. - Mango

Species: Mangifera indica L. - Mango

(National Plant Database. 2004.)

2.4 *Pelthophorum pterocarpum*

Pelthophorum pterocarpum (PP) possess a wide range of medicinal properties.PP which can been seen in Figure 2.4 or commonly known as Copperpod, Golden Flamboyant, Yellow Flamboyant, Yellow Flame Tree, Yellow Poinciana, Konda Chinta in Telugu, Perunkonrai in Tamil, Radhachura in Bangla. It is widely distributed in tropical South East Asia, from Sri Lanka in West, Indochina in the north, throughout Malaysia to northern Australia in the south. Originally this plant is located at sandy and rocky shores and sometimes found in limestone plateau of Malaysia. PP in Malay is known as Batai Laut or Jemerlang Laut. This ornamental tree has the ability to overcome grass trees and is rather wind-firm.

In conventional medicine, flowers are used as an astringent to cure or relieve intestinal disorders after pain at childbirth, sprains, bruises and swelling or as a lotion for eyes troubles, muscular pains and sores. A research states that 70% ethanolic extract of PPpossess hepatoprotective property where it may be attributed to the anti-oxidant principles of the extract (Biswas *et al*., 2009).

Figure 2.4: Picture of Pelthophorum pterocarpum plant

Peltophorum pterocarpa (DC.) Backer ex K. Heyne

Kingdom: Plantae-Plants

Class: Magnoliopsida-Dicotyledons

Subclass: Rosidae

Order: Fabales

Family: Fabaceae - Pea family

Genus: Peltophorum (T. Vogel) Benth. - Peltophorum

Species: Peltophorum pterocarpa(DC.)Backer ex K. Heyne - Yellow Poinciana (National Plant Database. 2004.)(Smith, 1985)

2.5 APOPTOSIS

Apoptosis is best explained as distinct morphology of dying cells. The term was created because it appeared as the picture of falling leaves from deciduous trees, called in Greek "apoptosis on the fact that the release of apoptotic bodies by dying cells resembled" (Kerr *et al*., 1972). In simple term, apoptosis is defined as a 'suicide' program which is activated within the cell, leading to fragmentation of the DNA, shrinkage of the cytoplasm, membrane changes and cell death without lysis or damage to neighbouring cells, and also a type of programmed cell death (PCD). Chromatin condensation and nuclear fragmentation were the major morphological characteristics that happen in apoptosis which accompanied by rounding up of the cell, reduction in cellular volume (pyknosis and retraction of pseudopodes) (Kroemer *et al*., 2005). Chromatin condensation begins at the periphery of the nuclear membrane, appearing like a crescent or ring-like structure. The chromatin later condenses until it splits inside the cell with an intact membrane, karyorrhexis. The plasma membrane is intact throughout the total process (Kroemer *et al*., 2005). Morphological features in apoptosis include membrane blebbing, modification of cytoplasmic organelles and a loss of membrane integrity. It is a normal phenomenon, occurring frequently in a multi-cellular organism. This cell death is very important to maintain the homeostatic balance of the body.

Apoptosis is very important in both physiological and pathological conditions. Pathological conditions can be described as anticancer drug induced cell death tumor, cell death that occurs in heart diseases such as myocardial infarction and cell death due to injurious agents like radiation, hypoxia and mild thermal injury. Morphological

changes in apoptotic cell death concerns both the nucleus and the cytoplasm are extremely similar across cell types and species (Antti & Kari 1999). Usually, several hours are required from the initiation of cell death to the final cellular fragmentation. Figure 2.5 explains the process where the cells undergo apoptosis and necrosis. Apoptotic cells will shrink and their chromatin will be condensed. Then, the cells budded and finally formation of apoptotic bodies takes place. While in necrosis cells, cells will swell, then there will be leakage in the cells, and the cell dies due to inflammation.

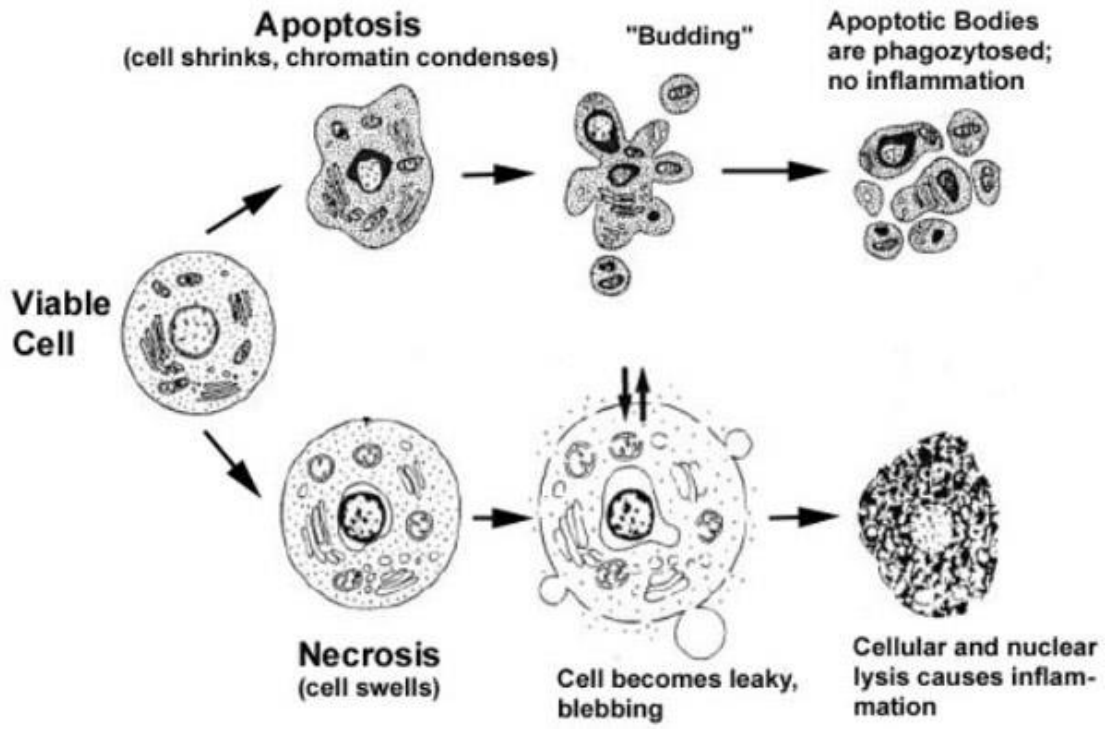

Figure 2.5: Hallmarks of the apoptotic and necrotic cell death process (Gewies, 2003)

Biochemical markers of apoptosis include activation of proteases termed caspases, cleavage of proteins and DNA and exposure of phosphatidylserine on the cell surface. It is very important to understand the mechanisms of apoptosis as it is very vital and might assist in improving the development of drugs to target the apoptotic genes and pathways. Caspases are very essential in the mechanism as they play role as initiators and executioners (Wong, 2011). There are 2 main pathways where caspases can be activated; extrinsic and intrinsic pathway. Extrinsic apoptotic pathway or also known as extra-cellularly activated starts when soluble tumor necrosis factor (TNF) family ligands TNF receptor (TNFR) apoptosis-inducing ligand (TRAIL), FasL, and TNF form primers that recognize and bind their cognate death receptors (Hengartner, 2000). Fas go through

conformational changes resulting in gathering of the death-inducing signalling complex, DISC. Fas then recruit Fas-associated death domain (FADD) through complementary death domains (DDs). FADD can recruit Caspase 8 through their complementary death-effector domains (DEDs). Recruitment of Caspases 8 to the DISC leads to its autoproteolytic cleavage. Active Caspase-8 then processes downstream effector caspases which subsequently cleave specific substrates resulting in cell death. Cells harboring the capacity to induce such direct and mainly caspase-dependent apoptosis pathways were classified to belong to the so called type I cells (Scaffidi *et al*., 1998).

Mitochondria also has a major role in the integration and propagation of death signals originating from inside the cell such as DNA damage, oxidative stress, starvation, as well as those induced by chemotherapeutic drugs via intrinsic pathway (Kaufmann & Earnshaw 2000). Usually apoptosis-inducing environment involve the interruption of the mitochondrial inner transmembrane potential ($\Delta\psi$) and permeability transition (PT). Osmotic mitochondrial swelling has been experimental by influx of water into the matrix with final rupture of the outer mitochondrial membrane causing the release of proapoptotic proteins from the mitochondrial intermembrane space into the cytoplasm (Loeffler and Kroemer, 2000). Cytochrome c, one the proteins that are released activates the apoptosome and therefore the caspase cascade, and also other factors such as the apoptosis-inducing factor AIF (Susin *et al.*, 1999).

2.6 CELL CYCLE

Homeostatis within the cells will be maintained and controlled by the cell cycle mechanism. This process will decide if the cell will experience cell proliferation, or undergo apoptosis or known as programme cell death (PCD). Cancer cells start to grow or the presence of these cells occurs when there is a failure in the cell cycle regulation. The fundamental characteristic of the cell cycle is to make sure that the replication of the DNA take place on time once during the S phase and those identical chromosomal copies are distributed equally to two daughter cells during Mitosis phase. Therefore, genes commanding these processes should not be targets of mutation, deletion, or amplification in cancer.

Cell cycle in an omnipresent and complex process that involves in proliferation of the cells, development or growth of the organism, regulation of DNA damage repair. Cell division occurs every 24 hours, where it includes four organized processes of cell cycle, which is, cell growth, DNA replication, distribution of duplicated chromosomes to daughter cells and division of cells. Cell division consists of two consecutive processes, mostly characterized by DNA replication and separation of replicated chromosomes into two separate cells. There are two main stages in cell division, mitosis (M), known as the process of nuclear division; and interphase, the interlude between two M phases, which is clearly illustrated in Figure 2.6. Stages of mitosis comprise of prophase, metaphase, anaphase and telophase. Review in Norbury and Nurse (1992) states that interphase cells simply grow in size, but different techniques revealed that the interphase includes G1, S and G2 phases. Replication of DNA takes place in a specific part of the interphase called S phase. G1 is a gap that happens during the cell cycle in preparing for DNA synthesis,

followed by S phase and followed by a gap called G2 during which the cell prepares for mitosis. G1, S, G2 and M phases are the fixed subdivisions of the standard cell cycle. Cells in G1 enter a resting state called G0. Cells in G0 account for the major part of the non-growing, non-proliferating cells in the human body (Vermeulen *et al*., 2003).

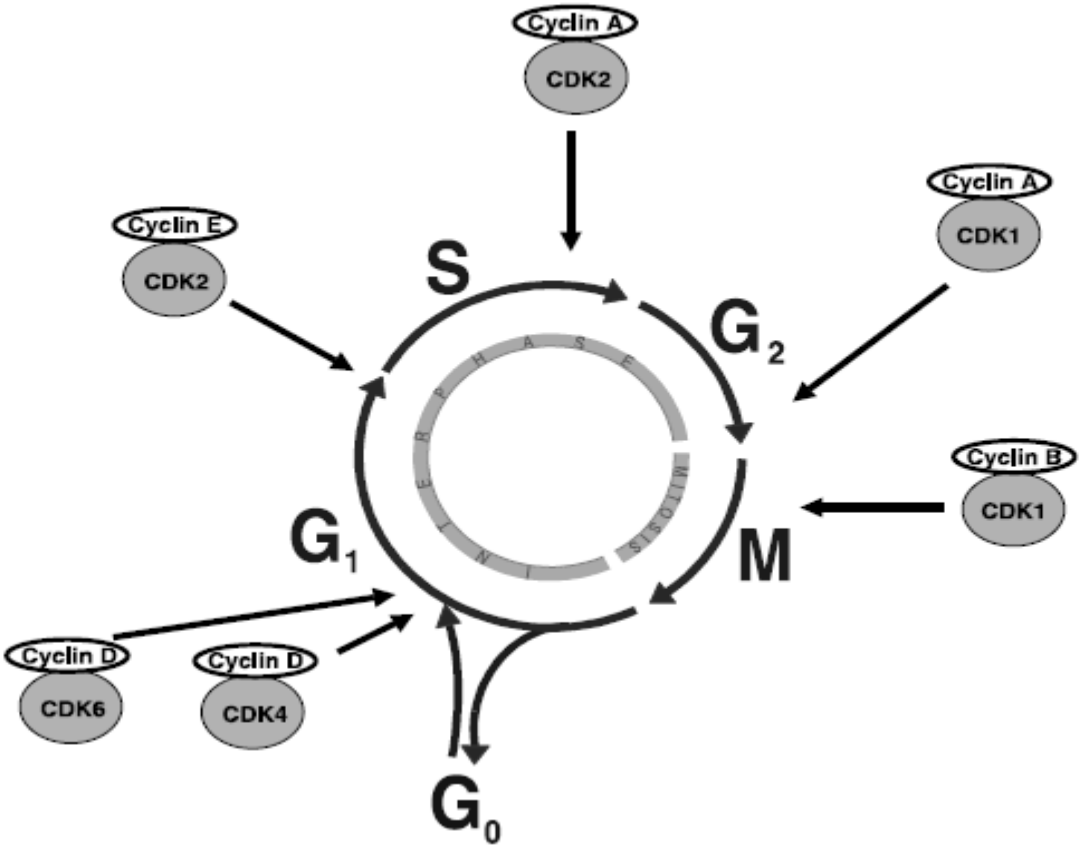

Figure 2.6: The stages of the cell cycle. The sites of activity of regulatory CDK/cyclin complexes are also indicated.

The conversion from one phase to another phase in cell cycle is an organized form and is regulated by various cellular proteins. Cyclin dependent kinases, CDK or also known as key regulatory proteins is from the family of serine/theornine protein kinases that are triggered at definite points in the cell cycle process. Up to date, there are