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Effect Of Oil Palm Phenolics (opp) On Pancreatic Cancer Cell Lines

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EFFECT OF OIL PALM PHENOLICS (OPP) ON PANCREATIC CANCER CELL LINES

by

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DEDICATION

Finally, I dedicate this thesis to my late grandparents who raised me and instilled in me the importance of hard work, perseverance, and kindness from a very young age.

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LIST OF ABBREVIATIONS AND ACRONYMS

AA	Amino acids
ATRX	ATP-dependent helicase ATRX, X-linked helicase II
BCL2	B-cell lymphoma 2
BxPC-3	Human pancreatic cancer cell line used in the study of pancreatic adenocarcinomas
CDKN2A	Cyclin-dependent kinase Inhibitor 2A
CT	Computerized tomography
Cxcl12	C-X-C Motif Chemokine Ligand 12
DAXX	Death-Domain Associated Protein
DNA	Deoxyribonucleic nucleic acid.
dNTP	Deoxynucleotide triphosphate
IL-12	Interleukin 12
KRAS	Kirsten rat sarcoma viral oncogene
MLH1	MutL Homolog 1
MPOB	Malaysian Palm Oil Board
MRCP	Magnetic Resonance Cholangiopancreatography
MRI	Magnetic Resonance Imaging
mTOR	Mechanistic target of rapamycin
MTS Assay	Cell proliferation assay
NEUROD1	Neuronal differentiation 1
NF- Ƙb	Nuclear factor- kappa-B
NKX2- 2	NK2 Homeobox 2

NR5A2	Nuclear receptor subfamily 5 group A member 2
OPP	Oil palm phenolic
Panc-1 cell	Human pancreatic cancer cell line isolated from a pancreatic carcinoma of ductal cell origin
PC	Pancreatic cancer
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
PPPD	Pylorus-preserving pancreaticoduodenectomy
RBPJL	Recombination signal binding protein for immunoglobulin Kappa J region
RNA	Ribonucleic acid
SMAD4	SMAD Family Member 4
TP53	Tumor protein p53
TRAF1/2	TNF receptor-associated factor 1

CHAPTER 1: INTRODUCTION

CANCER

Cancer is the abnormal and uncontrolled proliferation of cells in the body, leading to the formation of cell masses that are malignant and have the potential to invade other parts of the body through metastasis. The cell masses that form go through the six hallmarks of cancer to form malignant tumors and include avoiding programmed cell death and lack of proper cell division and growth signals [1]. Also, the cancer cells have an unlimited number of cell divisions due to the continued cell proliferation with the contrary signals. Normal cells have constant signals that dictate the cycles of cell division and differentiation and that initiate apoptosis, leading to controlled cell growth and division [1]. Similarly, the malignant cells promote the construction of blood vessels within the masses to enhance their blood supply and invade tissues while forming metastases [2]. The cancer cells can spread from their original sites through lymphatics to regional lymph nodes, through local spread or hematogenous routes to distant sites all over the body [2]. Nonetheless, the cancer cells establish themselves in given sites only as hypothesized by the Soil and Seed Hypothesis of Cancer Metastasis [2].

The different causes of cancer directly alter genes to cause deletion, point and insertion mutations at different levels within the cells. The various levels on which mutations occur can be chromosomal in which there can be losses or gains from chromosomes, nucleotide level or the silencing or activation of a microRNA that controls the expression of genes. The altered genes act as oncogenes that promote the proliferation of the cells. The progression of cancer, oncogenesis, is a multistep process through which normal cells are transformed into cancer cells: initiation, promotion, and progression [2]. Initiation is the irreversible changes in targeted somatic cells whereby the genome undergoes mutation. The changes at this level can affect cell behavior and

response, affecting its response to signals. However, the DNA repair mechanisms can repair damaged genomes in cells before the cell proliferates with the damaged DNA. Delava and Birnbaum note that the promotion stage involves the prolonged expression of the mutations that depends on the interaction of the cells with other oncogenic mutations and factors that are involved in gene expression [2]. The promotion results in enhanced cellular growth potential and the uncoupling of cell communication systems that limit their autonomy, development, and maintenance [2]. During the last stage, progression, there are successive changes in the cell masses that result in several malignant populations. The stage is accelerated by the continued exposure to carcinogenic stimuli or the selection pressures that favor the autonomous clonal derivatives [2]. The cancer cells proliferate, and the size of the malignant tumor increases, as more mutations occur, leading to increased heterogeneity of the cell population.

Metastasis is the spread of the cancer cells to other body parts. The progression of the malignant tumors results in loss of the cells' adherence property in which they detach from the mass of the cells and invades neighboring tissues [2]. Also, they enter the lymph and blood system and are transported to sites organs that are away from the primary location, forming distant metastases. Cancer has a poor prognosis and its treatment depends on its stage during diagnosis. The early stages are treated through surgery that entails the removal of the tumor and nearby cells that may have acquired the mutations. Late stages of the cancer are treated through radiation therapy which is the use of high energy X- rays and chemotherapy, the use of drugs to destroy the cancer cells. Targeted therapy is also used on particular cells, tissues, and proteins. However, the treatment for pancreatic cancer is still a challenge despite the advances that have been made in understanding its molecular progression.

Overview of Pancreatic Cancer

The pancreas is an organ in the abdomen that has both endocrine and exocrine functions. Its endocrine function is the production of insulin hormone that regulates the level of blood sugar while its exocrine function is the production and secretion of intestinal digestive enzymes, through ducts, that aid in the breakdown of fats, proteins, and carbohydrates.

Pancreatic cancer is caused by the abnormal and uncontrolled proliferation of cells in the pancreas, and more than half of the diagnosed cases are in people aged 75 years and above [5]. Notably, it is uncommon among people below 40 years of age [3]. It is aggressive and tends to spread silently and rapidly to nearby organs before a diagnosis is made, and as such, it has a poor prognosis [4]. Also, it is seldom detected in its early stages, although in individuals with a family history of cancer or pancreatic cysts, it can be detected early through screening [4]. The genetic events that occur in invasive ductal adenocarcinoma have been characterized, and exome sequencing has been conducted for the common types of pancreatic cancer [6]. The genes that mutate in a majority of the adenocarcinomas are KRAS, CDKN2A, TP53, and SMAD4 and these are associated with poor prognosis [5]. SWI/ SNF mutations and deletions occur in about 10 - 15% of adenocarcinomas and neuroendocrine pancreatic cancers have mutations of the DAXX, mTOR and ATRX genes [7]. Accumulation of the genetic changes in pancreatic cells leads to the multi-staged process of malignancy and mutations in the proto-oncogene KRAS, and tumor suppressors alter the cell cycle [5]. Alteration of the cell cycle affects apoptosis and controlled proliferation and leads to invasion and metastasis and inadequate treatment options.

The pathophysiology of the pancreatic cancers is different and exocrine cancers arise from precancerous lesions within the pancreas. Some neoplasms of the pancreas are curable lesions, and some do not always progress to malignant tumors [8]. For example, the pancreatic intraepithelial

neoplasia are microscopic abnormalities that are commonly found in autopsies of individuals who have not been diagnosed with cancer, although they have the potential to progress from low to high grade. The intraepithelial neoplasia contains faulty KRAS genes in grade 2 and in grade 3, other genes such as CDKN2A, P53, and SMAD4 are damaged [8]. The intraductal papillary mucinous neoplasms (IPMNS) are macroscopic lesions found in 2% of add adults and have a 25% risk of developing into invasive cancers. They also have KRAS gene mutations. Wolfgang et al. add that the pancreatic mucinous cystic neoplasms are commonly found in women, and although benign, they have the potential to become malignant [5]. The last type of neoplasia is the intraductal tubulopapillary neoplasm that accounts for about 1-3% of all the pancreatic neoplasms. Rooney and Shi note that their histological diagnosis is not easy, and they have a 50% chance of becoming invasive [9].

Prevalence and Statistics

There are no tools to diagnose the disease in its early stages when treatment through surgical removal of the tumor is possible and as such, its prognosis is poor [10]. Notably, pancreatic cancer has high fatality rates such that the incidence rates are similar to the mortality rates [11]. Over almost 40 years, the survival rate of pancreatic cancer has not improved due to the late stage detection and 91% of the diagnosed patients die within 5 years of diagnosis. Compared to other cancers, it is rare and rates twelfth after breast, lung, and bronchus, prostate, colorectal, melanoma of the skin, bladder, non-Hodgkin lymphoma, kidney, renal pelvis, leukemia and uterine cancers [8]. However, it is the third leading cause of cancer-related deaths in the US after breast cancer, although it is expected to surpass colorectal cancer and become the second leading cause. The number of new cases and deaths per 100, 000 people is 12.5 and 10.9 respectively [8]. The National

Cancer Institute notes that approximately 1.6% of people will be diagnosed with pancreatic cancer at some point in their lifetime [8].

In 2014, an estimated 64, 668 people were living with pancreatic cancer in the US and in 2017, the estimated number of new cases was 53670 with 43090 deaths [8]. This accounts for 3.2% of all new cancer cases in the US. Notably, the disease is common with increasing age and statistics indicate that it is more prevalent in men than women. The National Cancer Institute indicates that there 14.2 and 11.1 men and women respectively in 100, 000 people, based on the cases reported in 2010- 2014 [8].

Molecular Pathways

Pancreatic cancer is thought to be caused by mutations in genes that make them oncogenes or turn off tumor suppressor genes, leading to the uncontrolled growth and division of cells. The gene mutations can either be inherited or acquired, and some of the acquired mutations occur on different genes including p16, TP53, P16/ CDKN2A BRAF, KRAS, and SMAD4. Inherited mutations occur in genes such as BRCA1/2, MEN1, NF1, and KRAS. The KRAS gene encodes a GTPase that regulates downstream signaling of growth factor receptors, and its mutations are observed in early pancreatic intraepithelial neoplasia [12]. The P16/ CDKN2A gene encodes a cell cycle regulator that suppresses tumors and is observed in more than 90% of all pancreatic cancer cases [12]. Mutation in the P53 gene is associated with cellular responses to cytotoxic stress, as it contributes to both cell cycle arrest and apoptosis. SMAD4 tumor suppressor gene encodes a transforming growth factor beta signaling pathway and studies have indicated that its presence in early mutations is associated with poor prognosis. Mismatch mutations on the mutL homolog 1 (MLH1) and cationic trypsinogen, serine, protease, and trypsin genes have also been observed in exocrine pancreatic cancers, and they are associated with a high impact of malignant progression

than the initiation of cancer [12]. Mutations on the B- Raf proto-oncogene serine/ threonine kinase (BRAF) that encodes amino acids that belong to mitogen-activated protein kinases that activate the extracellular signal-regulated kinases in the Mitogen-activated protein kinase (MAPK) signaling pathway have also been noted.

The MAPK signaling pathway communicates signals from cell surface receptors to the DNA in the nucleus and mutations make the proteins to remain in 'on' or 'off' position that is key to the development of various cancers including pancreatic cancer. Phosphatidylinositol- 4,5 - biphosphate 3- kinase (PI3K) signaling pathway has also been observed in pancreatic cancer, and it mediates the growth and survival of cells through downstream substrates such as protein kinases that regulate multiple cellular processes [12]. Constant activation of the PI3K is closely linked to the carcinogenesis. Panc-1 cell line has a role in the development of pancreatic cancer as indicated by studies that characterized the genotype and phenotype of the cell line [12].

The nuclear factor- kappaB (NF- ƙB) pathway is thought to be involved in the progression of cancer, as its activation has been noted in solid tumors. NF- ƙB family consists of five transcription factors that regulate gene expression during apoptosis, inflammation, replication, and tumorigenesis [13]. Transcription factors such as TRAF1/2 and BCL2 target anti-apoptotic genes and have been implicated in the progression of pancreatic cancer and other cancers such as prostate, lung, breast, and cervical [12]. These factors form distinct protein complexes that bind to DNA sequences at promoter regions of the responsive genes. Their involvement in the development of cancer is due to the inflammatory micro-environment that is created and the oncogenic mutations. Also, the activity of NF- ƙB promotes the proliferation of tumor cells, angiogenesis and suppresses apoptosis while inducing the transition of epithelial-mesenchymal transition that encourages metastasis of the malignant cells [12]. Xia, Shen, and Verma add

however that the suppression of the NF- ƙB pathway in myeloid and malignant cells leads to the regression of tumors and as such, it can be a therapeutic target [13]. Inflammation is a key defense mechanism in innate immunity, and when prolonged, it leads to the damage of tissues, degenerative and autoimmune diseases and cancers, as it increases cellular stress, accumulation of damaged DNA and inflammatory factors are recruited. The accumulation of the inflammatory cytokines such as TNF and IL- 1/ 17 at sites promotes a pro-tumorigenic microenvironment.

Causes of Pancreatic Cancer

Tobacco Use

Smoking is a cause of pancreatic cancer, and the American Cancer Society notes that individuals who smoke are likely to develop cancer more than those who do not. Other tobacco products such as cigars and pipes also increase the risk of one [14]. The components in tobacco such as nicotine and nitrosamines are carcinogenic substances that stimulate the development of pancreatic cancer through the promotion of inflammation and fibrosis. Together with genetic factors that predispose one to the cancer, inflammation, and fibrosis leads to the inhibition of apoptosis while it stimulates the proliferation of cells.

Family History

Genetics and positive family history are associated with an increased risk of pancreatic cancer. The susceptibility of one developing the cancer is high if at least two first degree relatives have been diagnosed with cancer. Some gene mutations that are inherited such as point and mismatch mutations to genes that are affected in the cancers lead to its development.

Overweight, Diet and Obesity

Regular intake of foods that are high in fat can lead to the development of pancreatic cancer. Also, carrying extra weight around the waistline is a risk factor for people who are not obese [15]. Diet

also plays an important role in increasing the risk of developing pancreatic cancer and red meats and processed foods such as sausages and bacon elevate the risk, as well as low fruits and vegetables. Plausible mechanisms that link obesity to pancreatic cancer include the promotion of inflammatory factors and hormonal effects that are associated with adiposity and the energy-balance that is related to increased exposure of carcinogens as a result of high-calorie intake and reduced physical activity.

Pancreatitis

Chronic pancreatitis, long-term inflammation of the pancreas, is associated with an elevated risk of developing cancer, particularly in individuals who smoke [14]. Chronic pancreatitis may be due to inherited gene mutations. Also, it is associated with inflammatory factors such as cytokines and interleukins that promote a pro-tumorigenic environment that increases the proliferation of cells while hindering apoptosis.

Cirrhosis of the Liver

This is the scarring of the liver due to damage to the liver from heavy use of alcohol and studies have shown that it is associated with the development of pancreatic cancer [14]. Liver cirrhosis is associated with fibrosis that affects the liver parenchyma and blood flow. This could lead to mutations in the cells that are affected and when associated with inflammation that can occur, tumors that have a potential of malignancy can be formed.

Risk Factors for Developing Pancreatic Cancer

I.   Diabetes

Insulin resistance that characterizes diabetes is thought to be a risk factor to the development of pancreatic cancer, although the mechanisms are not clear. Studies show that people who have

had diabetes for several years have an elevated risk of developing pancreatic cancer [15]. Besides, sudden development of diabetes in adulthood can be a sign of pancreatic cancer.

II. Age

People aged 65 years and above have a high risk of developing cancer, and the average diagnosis age is 71 [15]. In the UK, more than 9 out of 10 people who have been diagnosed with pancreatic cancer are aged over 60 years [15]. There are biological patterns of aging that are associated with the development of cancers. For instance, it is marked with the progressive declining of the functioning of cells and tissues and an increase of hyperplasia and cell senescence.

III. Long-term Inflammation of the pancreas

The inflammation of the pancreas, chronic pancreatitis, is linked to an increased risk of pancreatic cancer, especially in people who smoke as it sometimes occurs due to an inherited gene mutation that can affect the growth and proliferation of pancreatic cells. Inflammation of the pancreas leads to the accumulation of inflammatory and tumor factors such as TNF that promote a pro-tumorigenic environment that leads to increased cell division.

IV. Race and Gender

Black people are more likely to develop pancreatic cancer than Asian, whites or Hispanic people. African Americans are more susceptible than other races in the U.S., although this is attributed to poor socioeconomic factors and cigarette smoking [14]. Similarly, the Jewish race has the breast cancer gene BRCA2, and this is a risk factor in the development of the condition.

V. Infection with Hepatitis Viruses and bacterial infections

These viruses infect the liver and studies indicated that previous infection with hepatitis B infection was twice as common in people with pancreatic cancer as in those without [15]. Infection by the Helicobacter pylori bacteria leads to the inflammation of the stomach and ulcers and is

associated with an elevated risk of developing stomach and pancreatic cancers, although the risk for the former is higher than the latter [15]. The increased risk after the infection is due to reduced immune system functioning and when combined with the inflammation that leads to the accumulation of inflammatory and tumor markers, the risk of developing the cancer increases.

VI. Exposure to Chemicals

Exposure to carcinogenic chemicals and substances such as benzene, pesticides, asbestos, certain dyes, and petrochemicals increases the risk of one developing cancer [15]. Some of these chemicals lead to point mutations in genes that are linked to pancreatic cancer.

VII. Rare inherited conditions

People with rare inherited conditions such as hereditary breast and ovarian cancer syndrome, familial atypical multiple mole melanoma syndromes (FAMM), familial pancreatitis, Lynch syndrome, Peutz- Jeghers syndrome and the Von Hippel- Lindau syndrome have a higher risk of developing cancer later in life. These inherited conditions result due to mutations in particular genes that are linked to pancreatic cancer, including BRCA1/2, PRSS1, MLH1/2, STK11 and VHL genes [14]. Besides, the neuroendocrine pancreatic tumors can be caused by genetic syndromes such as neurofibromatosis type 1 and multiple endocrine neoplasia types 1 (MEN), due to mutations of NF1 and MEN1 genes that increase the risk of one developing tumors of the parathyroid gland, islets of the pancreas and the pituitary gland [14].

VIII. History of Cancer

Individuals who have had cancers of the breast, womb, colorectal, cervix, lung or testicles are more susceptible to develop pancreatic cancer. The risk is due to common genetic variations such as mutations on BRCA2/1.

Types of Pancreatic Cancer

Pancreatic cancer occurs in two forms, adenocarcinoma, and pancreatic endocrine tumors. The former accounts for 85% of all cases, while the latter constitutes 5% of all pancreatic cancer cases [14]. A 2016 study in which the researchers evaluated genes in 456 pancreatic ductal adenocarcinomas and conducted expression analysis led to the classification of the adenocarcinomas into four subtypes: squamous, pancreatic progenitor, immunogenic and aberrantly differentiated endocrine exocrine (ADEX) [16]. Squamous adenocarcinomas are enriched with TP53 and KDMA mutations while pancreatic progenitors hade FOXA2.3, MNX1, and PDX1 gene mutations. The Immunogenic adenocarcinomas have pathways that take part in the suppression of acquired immune functions while the aberrantly differentiated endocrine, exocrine adenocarcinomas (ADEX) have KRAS genes, exocrine genes NR5A2 and RBPJL and endocrine NEUROD1 and NKX2- 2 differentiation.

The types of pancreatic cancer can also be classified into two general groups: exocrine and neuroendocrine cancers [17]. The exocrine component of the pancreas, the digestive enzymes, account for a large percentage of them while hormone-producing endocrine tissues of the pancreas account for a small percentage of the pancreatic cancers. Pancreatic adenocarcinoma is the common type of exocrine pancreatic cancer, and it begins in the ducts that carry secretions such as the enzymes and bicarbonates and is therefore called pancreatic ductal adenocarcinoma [17]. Acinar cell carcinoma of the pancreas is another form of exocrine cancers, and it arises in the clusters of cells that produce the enzymes, and its manifestation leads to overproduction of the enzymes leading to symptoms such as skin rashes and joint pain. Cystadenocarcinomas are also exocrine cancers of the pancreas and pancreatoblastoma is a rare form that affects children, although it has a good prognosis. Other forms of pancreatic exocrine cancers include

adenosquamous carcinomas, signet ring cell carcinomas, colloid, and hepatoid carcinomas and undifferentiated carcinomas and pancreatic mucinous cystic neoplasms [17].

Neuroendocrine pancreatic cancers are a group of tumors that arise from the neuroendocrine cells in the pancreas that integrate the nervous and endocrine systems. The neuroendocrine pancreatic cancers are grouped into functioning and non- functioning types, depending on the level of hormones they produce [18]. The former secrete various hormones including insulin, glucagon, and gastrin into the bloodstream in large quantities resulting in low blood sugar levels, although their diagnosis is easier than the latter that are commonly diagnosed in later stages. Common types of pancreatic neuroendocrine cancers are insulinomas and glucagonomas [18]. The glucagonomas produce glucagon hormone that leads to skin rashes called necrolytic migratory erythema. Notably, there are genetic differences in the manifestation of exocrine and neuroendocrine pancreatic cancers [7]. For example, KRAS mutation is absent in neuroendocrine pancreatic cancer while it is present in exocrine cancer. The hereditary MEN1 gene mutations in neuroendocrine pancreatic cancer give rise to the MEN1 syndrome in which primary tumors occur in one or two endocrine glands that secrete the hormones. Thakker et al. note that individuals who are born with a mutation of the MEN1 gene are at an elevated risk of developing the neuroendocrine pancreatic type of cancer [20].

Symptoms

The symptoms of exocrine and neuroendocrine pancreatic cancers are different, and during the early stages of the disease, there may not be any associated symptoms, as they begin to show in the late stages after it has metastasized. Exocrine pancreatic cancers are characterized by jaundice and related symptoms such as dark urine and itchy skin, belly and back pain, weight loss and reduced appetite, nausea and vomiting, enlargement of the liver or gallbladder, blood clots,

abnormalities of fatty tissues, and diabetes [19]. Jaundice, the buildup of bilirubin, begins when the pancreatic ducts are blocked by the tumors. The pain one experiences emanate from the tumor pressing on nearby organs and nerves surrounding the pancreas, leading to backaches. The enlargement of the liver is because the cancer cells spread to it and the gallbladder enlarges due to the buildup of bile [20]. Deep vein thrombosis can also occur resulting in pain, swelling, and redness of the affected legs and if the clot travels to the lungs, it affects the breathing ability of one.

In neuroendocrine pancreatic tumors, the excretion of excess hormones into the bloodstream results in varying symptoms that vary according to the hormones produced. For instance, gastrinomas increase the acidity in the stomach leading to ulcers, nausea, pain, and loss of appetite, and damage to the intestinal lining [20]. The glucagonomas elevate the blood sugar level leading to thirsty feelings, urge to urinate and weight loss, while insulinomas lower the blood sugar level leading to confusion, sweating spells, rapid heartbeats and weakness of the patient. Somatostatinomas affect the production of somatostatin hormone that regulates other hormones, and it results in reduced appetite, loss of weight, diarrhea, nausea, and jaundice [20]. Metastasis of pancreatic cancer leads to enlargement of the liver, jaundice, pain, and loss of appetite. The symptoms presented by the disease are common in other conditions. For instance, jaundice is also caused by hepatitis, gallstones and liver and bile duct diseases.

Staging of Pancreatic Cancer

Staging of cancer describes the extent of its spread and this is used to guide its treatment after the classification. The staging system by the American Joint Committee on Cancer is often used, and it is based on three factors: the extent of the tumor (T) including its size and if it has metastasized, the spread to nearby lymph nodes (N) and its metastasis to distant organs such as the

lungs and bones (M) [21]. Notably, the staging utilizes the pathologic staging that is determined through the examination, imaging, and screening of the patient to indicate the size of the tumor, if it has affected lymph nodes or metastasized[21]. The table below describes the staging in detail, as adapted from Dragovich, 2017) [21] (Table 1-1 Stages of pancreatic cancer).

The prognostic factors in the staging of pancreatic cancer include the tumor grade that describes the look of malignant tumors under a microscope. G1 indicates that the cancer is similar to normal pancreas tissues, grade 2 shows that it is abnormal and grade 3 means that the tumor falls in between. The American Cancer Society notes that low-grade cancers have a tendency of growing and spreading at a slow speed than the high-grade pancreatic cancer that also has a poor prognosis.

Diagnosis of Pancreatic cancer

I.   Physical Examinations and Medical History

During physical examinations, the doctor examines the belly to determine if the liver and gallbladder are swollen and examines for symptoms such as jaundice. Medical history is important as the disease is genetic and individuals whose first relatives are affected have an elevated risk of developing cancer.

II.   Laparoscopy and Biopsy

Laparoscopy and biopsy tests are used in its diagnosis [22]. The examination of pancreatic tissues under a microscope, biopsy, is a definite method that diagnoses pancreatic cancer, and it can be done in several ways including percutaneous needle biopsy, endoscopic or surgical biopsy.

III.   Blood Tests

There are also blood tests for exocrine pancreatic cancers, and these include liver function tests, tests for tumor markers and kidney and bone marrow function tests [23]. Liver function tests

assess the level of bilirubin in the blood and define the functioning of the liver. Tumor markers such as CA 19- 9 and carcinoembryonic antigen (CEA) can be used to diagnose pancreatic cancer in patients, although they are not accurate as high levels of tumor markers have been noted in individuals without the cancer [23]. CA 91- 9 biomarker is useful in symptomatic patients and is used as a prognostic factor, although its levels are normal in the early stages of the disease and are therefore not suitable [24]. The carcinoembryonic antigen has low sensitivity, and its elevation is common in other malignant and benign conditions and is therefore not specific to pancreatic cancer [24]. Blood tests for neuroendocrine pancreatic tumors indicate the levels of particular hormones such as insulin, glucagon, gastrin, and somatostatin. They also determine the levels of glucose, chromogranin and for insulinomas, C- peptide levels are tested. Serotonin levels and related chemicals such as 5- HIAA and 5- HTP are tested for carcinoid tumors as they lead to increased production of the hormone.

IV.   Imaging Modalities

Diagnosing pancreatic cancer takes a long time, mostly because there are few or no symptoms during the early stages and currently, there are no established tools that are used for its early detection. It is primarily diagnosed through imaging techniques such as abdominal ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI), Positron Emission Tomography (PET scan), endoscopic ultrasound (EUS) and endoscopic retrograde cholangiopancreatography (ERCP) [22]. The imaging tests give information on the size of the tumor, if the lymph nodes have been affected and if there are distant metastases, and the information acquired guides the treatment plans. Tummala, Junaidi, and Agarwal note that imaging is also non- invasive, and it enables the characterization of the cancer tumors [22]. However, these

diagnostic methods are unreliable in the detection of cancer in its early stages and small lesions that can be treated.

V. Abdominal Ultrasound (US)

Abdominal ultrasound is an imaging modality that does not have contrast associated effects and is conducted to rule out choledocholithiasis in patients while establishing biliary dilation [22]. Its accuracy is 50% as adipose tissues, and the results can be affected by overlying bowel gas and discomfort of the patient [22].

VI. Computerized Tomography (CT)

Computerized tomography (CT) gives cross-sectional images of the pancreas, and its accuracy is higher than that of US. Non- contrast CT evaluates the pancreas although its sensitivity and specificity are low as the pancreatic tumors are hypovascular and are better viewed on contrast imaging modalities [22]. CT with intravenous contrast has fast image acquisition, thin slice cuts, and high image resolution, allowing better visualization of the pancreatic adenocarcinomas. The high image resolution is important in the early detection of cancer tumors and accurate staging [22]. Tummala et al. note that the hypervascularity of the pancreatic ductal adenocarcinoma leads to poor enhancement compared to the surrounding parenchyma in the early phase of contrast CT and this enhances gradually with delayed images. CT angiography is used in preoperative staging and assessment of the tumors and is done by bolus administration of iodinated nonionic contrast [22].

VII. Magnetic Resonance Imaging (MRI)

MRI imaging is done with intravenous administration of contrast material although it does not have a significant diagnostic advantage over contrast-enhanced CT [24]. Nonetheless, MRI is better in the characterization of the lesions, and it gives direct radiological evidence that aids in

the diagnosis of pancreatic cancer [22]. Magnetic Resonance Cholangiopancreatography (MRCP) is a useful modality in diagnostic assessment of the disease as it creates 3D images of the pancreas, liver parenchyma, and the vascular structures and is, therefore, better than CT in the evaluation of the bile ducts and identification of intrahepatic mass lesions [22]. Also, MRCP is used in the distinction of chronic pancreatitis and pancreatic adenocarcinoma.

VIII.   Positron Emission Tomography Imaging (PET)

The use of Positron emission tomography imaging (PET) in the diagnosis and staging of pancreatic cancer and differentiation of malignant from benign tumors remains uncertain as it is performed after CT. Singer et al. report that the PET does not identify small metastases within the peritoneum area, and the liver and therefore, its accuracy is low [25].

Endoscopic Retrograde Cholangiopancreatography (ERCP). Further, the use of Endoscopic Retrograde Cholangiopancreatography (ERCP) in pancreatic cancer is preferred during surgery due to low resource utilization. The modality combines the use of endoscopy and fluoroscopy to diagnose pancreatic cancer in the ducts. Endoscopic ultrasound-guided fine needle aspiration is used in the diagnosis and staging of pancreatic cancer, and its sensitivity is 80- 95% [26]. It is useful in the evaluation of patients with inconclusive findings from CT and MRI. Contrast-enhanced CT and MRI are more preferred in the diagnosis of pancreatic cancer due to their high sensitivity and accuracy [27].

IX.   Treatment

Pancreatic cancer has a poor prognosis. The 5-year survival rate is less than is 7.2% for all types although survival may depend on the stage of diagnosis [28]. Diagnosing pancreatic cancer takes a long time, mostly because there are few or no symptoms during the early stages. Additionally, the cancer is associated with high metastasis rates rendering treatment difficult. The

treatment of pancreatic cancer depends on the stage of diagnosis, and early staged cancers are treatable.

a) Surgical Treatment

Surgery is performed when the tumor is small and has not spread to the neighboring organs and lymph nodes [28.] The surgery includes the removal of all or part of the pancreas although few patients undergo surgery as the cancer is diagnosed in late stages. Surgery can be combined with chemotherapy and radiation therapy through adjuvant therapy or neoadjuvant whereby they are used to reduce the size of the tumor before surgery. During the surgery, the margins of the removed are also removed to eliminate any unnoticed cancer cells of the healthy tissues [29]. Notably, there are different types of surgery that are performed depending on the purpose of the surgery, and these include laparoscopy, total pancreatectomy, distal pancreatectomy and pylorus-preserving pancreaticoduodenectomy (PPPD). Laparoscopy is performed to assess the size and extent of the spread while pancreaticoduodenectomy is carried to remove the head of the pancreas if the tumor is localized there. During pancreaticoduodenectomy, some parts of the stomach, nearby lymph nodes, and intestines are removed to get rid of the margins. Distal pancreatectomy is carried out to remove the body and tail of the pancreas while total pancreatectomy is conducted to remove the entire organ [29]. Surgeries to remove tumors that affect nearby blood vessels are also carried out by specialists and reconstructed in select patients.

b) Radiation Therapy

Radiation therapy is the use of high energy x- rays that destroy cancer cells and in pancreatic cancer, external beam radiation therapy is used. Conventional radiation therapy or stereotactic body radiation are also given in pancreatic cancer patients [30]. Stereotactic body radiation is used to provide localized treatment, and it requires few treatment sessions, unlike the

conventional radiation therapy that needs daily treatment of low radiation doses. The procedure reduces the likelihood of the recurrence of cancer and prolongs the lifespan of a patient. However, one experiences fatigue, nausea, loose bowel movements, and skin rashes after and during the treatment sessions.

c) Chemotherapy

Chemotherapy is the use of drugs to destroy cancer cells by stopping their division and growth. The therapy can be combined with radiation therapy to treat nearby metastases and to reduce the risk of recurrence [30]. It can be administered in a systemic way, intravenously, to reach cancer cells throughout the body or orally. First line chemotherapy is used in patients with locally advanced or metastatic pancreatic use while second-line chemotherapy is used in refractory cancer. The side- effects of chemotherapy include loss of appetite, nausea, vomiting, general weakness, diarrhea, and gastrointestinal problems and hair loss [30]. The decrease in the number of white blood cells and platelets increases the susceptibility of the patients to infections and loss of blood in minor injuries respectively.

d) Targeted Therapy

Targeted therapy is also used in the treatment of pancreatic cancer, and this therapy targets particular genes, cells, tissues and proteins that contribute to the uncontrolled growth of the cells [30]. The treatment reduces damage to healthy cells and tissues, and it requires pre-treatment tests that guide the therapy. For instance, erlotinib drug blocks the epidermal growth factor receptor (EGFR) that enables the growth and spread of the cancer cells. The treatment of neuroendocrine pancreatic cancer may include different approaches to the treatment of exocrine cancers [31]. Some of the cancers are identified incidentally during the imaging for other purposes. Palliative

care is given to patients with advanced pancreatic cancer, and it focuses on the treatment of symptoms such as pain and nausea and improving the quality of life of the patients.

e) Ablation Treatments for Pancreatic Cancer

Ablation is the treatment that destroys the pancreatic tumors using heat or cold. It is used to treat pancreatic cancer that has not spread to other organs like the liver, although it is not effective when used on its own, as they are more likely to relieve the symptoms [32]. In Pancreatic neuroendocrine tumors, ablation improves symptoms and prolongs the lives of individuals. In exocrine pancreatic cancer, however, the treatment is used less often in this type of cancers although it can be used when they have not spread [32]. There are different types of ablative treatments and they are; radiofrequency ablation (RFA), microwave thermotherapy and cryosurgery (cryotherapy/ cryoablation). Radiofrequency ablation uses high-energy radio waves that destroy the tumor cells. Microwave thermotherapy uses microwaves to heat and destroys the tumor while cryosurgery destroys the tumors by freezing. During the cryosurgery, cold gas is passed through a probe to freeze the tumor cells and it can be used on large tumors [32]. There are however side effects of ablation treatments and they include internal bleeding, abdominal pain, and infections, although serious complications are uncommon [32].

f) Embolization of Pancreatic Cancer

During this type of treatment, a doctor injects substances into arteries, to block blood flow to the cancer cells leading to their death [32]. Embolization can be used on large tumors when ablation is impossible and there are three main types of the therapy; arterial embolization, chemoembolization, and radioembolization. Arterial embolization (transarterial embolization) is the use of a catheter to inject small particles to the artery feeding the tumor. During the treatment, a dye is injected into the blood to monitor the path of the catheter with angiography.

Chemoembolization is a combination of chemotherapy and embolization and tiny particles that give off a chemotherapy drug are used to block the arteries that supply blood to the tumor [32]. Radioembolization combines radiotherapy with embolization and it is done by injecting small radioactive beads into the artery and these lodges in blood vessels near the tumor where they give off small amounts of radiation to the tumor site [32]. The side effects for embolization are nausea, pain, fever, and risk of blood clots in nearby blood vessels, although serious complications are uncommon.

The treatment for pancreatic cancer remains a challenge despite the advances that have been carried out in the understanding of the molecular mechanisms that underlie its development and progression. Vaccaro et al. note that researches that will enable the targeting of particular molecular pathways such as the VEGF- mediated angiogenesis, EGF receptor axis, MMPS, and mutant KRAS pathway and downstream signaling cascade, IGRF and embryonic pathways are underway [33].

Treatment outcomes

Pancreatic cancers and other exocrine cancers have a poor prognosis as they are discovered in advanced stages when cancer has metastasized and is locally advanced. Neuroendocrine pancreatic cancers generally have a better outcome than the exocrine cancers [32]. They have an average five- year survival rate of 16% unlike the 1% rate of exocrine cancers [32]. The survival rates of patients with locally advanced and metastasized carcinomas of the pancreas can be enhanced through the treatments and over the years, the survival rate of the patients has increased from 2% in 1977 to 6% in 2009. When the cancer is diagnosed in early stages, the survival rate is higher [32]. Pancreatic cancer has an increasing incidence, and with a few curative options and

poor prognosis, there is a need to have alternative and effective therapies, while enhancing the ability for the early detection of cancer.

Nutrition and Cancer

Secondary plant metabolites such as phenolic phytochemicals have important biological functions such as the protection of plants against physiological and environmental stress. In the recent past, the health effects of dietary phenolic compounds have attracted the attention of nutritionists, and their diversity and structural complexity are thought to enhance the biological importance [34]. The phenolic compounds include tocopherols, carotenoids, curcumin, vitamin C, beta-carotene, lycopene, catechins, and resveratrol. A study by Sambanthamurthi et al. to describe the effects of oil palm phenolics on degenerative diseases on animal models showed that the phytochemicals protected against various aspects of metabolic syndrome and type 2 diabetes [34]. In the study, the researchers inoculated the mice with tumors, and they noted that the oil palm phenolics protected the animal models against the progression of cancer [34]. Studies that have been conducted before indicating that the phytochemicals have anti-tumor molecular mechanisms and they may have several important disease outcomes on chronic conditions. In another study, Ji et al. evaluated the apoptotic, anti-invasive properties and antiproliferative abilities of the oil palm phenolics on pancreatic cancer cells and studies through cytoplasmic histone DNA fragmentation and matrix invasive assays [35]. The results obtained indicated that oil palm phenolics (OPP) have anti-tumor effects, giving evidence that the phenolics have the potential for therapeutic use. The oil palm phenolics have also been shown to have anti-inflammatory properties that suppress the progression of cancer tumors as inflammation links oxidative stress to chronic diseases [34]. Potential Role of Oil Palm Phenolics in Cancer Prevention and Treatment.

The oil palms [Elaeis (from Greek, meaning "oil")] comprise two species of the Arecaceae, or palm family that are used in commercial agriculture in the production of palm oil. Elaeis guineensis is native to West and Southwest Africa, found mostly between Gambia and Angola, while Elaeis oleifera is native to tropical Central and South America. In addition to oil, the palm fruit is a rich source of water-soluble compounds, which consist mainly of phenolic acids [36], thus, the water-soluble fraction is referred to as oil palm phenolics (OPP).

Due to their high redox potential, phenolics which comprise of an aromatic ring, are important antioxidants, acting as strong reducing agents, metal chelators, singlet oxygen quenchers, and hydrogen donors. Its antioxidative component helps it to counter the oxidative stress exerted by high temperature and intense sunlight. In recent studies, the antioxidant properties of OPP have been shown to be of great importance in counteracting the progression of chronic diseases such as cardiovascular disease and cancer in both mice human model [37].

Growing evidence points towards the health benefits of OPP, in studies where it has been assessed for positive bioactivities. In tumor-inoculated mice, OPP was proven to protect against the progression of certain cancers. Microarray studies on the tumors showed differential transcriptome profiles that suggest anti-tumor molecular mechanisms involved in OPP action [38]. Prooxidants such as reactive oxygen species (ROS) are known to play a role in triggering chronic diseases. An example is the hydroxyl radical that can lead to lipid peroxidation, DNA bases modification or protein damage, in turn, causing tissue damage, chronic diseases, and aging. Additionally, to oxidizing macromolecules such as DNA in the body, they also regulate gene expression, affecting two well-defined transcription factors, the nuclear factor kappa-B, and activator protein-1, resulting in inflammation. Inflammation, therefore, links oxidative stress with chronic diseases [39]. OPP has been found to have a higher antioxidant activity in vitro as

compared to other vitamin antioxidants. Phenolic compounds have been reported to demonstrate anticarcinogenic, antimutagenic, anti-inflammatory, antimicrobial, antiproliferative, antiviral and vasodilatory actions. Also, OPP tends to help in sensitizing tumor cells towards chemotherapy, and when used together with selected chemotherapeutic agents, it enhances cytotoxicity in cancer cells [40].

Chemical Properties of Oil Palm Phenolics

Phenolic acids are a large family of secondary metabolites having hydroxyl benzoic or hydroxyl cinnamic structures. They commonly occur as free acids while their glycosides, esters and bound complexes play an important role in plant resistance to oxidative stress, pathogens, allelopathy and herbivores, and also help in plant growth regulation [41]. Oil palm phenolics (OPP) include three major compounds, namely, p-hydroxybenzoic acid, protocatechuic acid, and caffeoyl-shikimic acid isomers. Phenolic compounds exhibit a wide range of biological and physiological properties due to their ability to act as antioxidants, free radical scavengers, and chelators of divalent cations. The antioxidant activity of phenolic compounds is influenced by several factors, reducing potential, polarity, position, and degree of hydroxylation, solubility, and stability of the phenoxy radical. In phenolic compound structures, of primary importance in determining their antioxidant activity is the position and degree of hydroxylation [42]. P-Hydroxybenzoic Acid is used as a chemical intermediate for synthetic drugs and in pharmaceuticals. A crystalline derivative of benzoic acid, it is a crystalline powder; slightly soluble in water, soluble in hot alcohol, either; it has a melting point of between 215 - 217 °C, the molecular weight of 138.12 [g/Mol], and a specific gravity of 1.48. It also contains both hydroxyl and carboxyl group at para-position, which react with either acid or alcohol [43].

Protocatechuic acid, an aromatic carboxylic acid containing carboxyl group bonded directly to the benzene ring, is a crystalline organic compound; melting at 122°C (starting sublime at 100°C); boiling at 249°C; slightly soluble in water and has a molecular weight of 154.12 [g/Mol]. It occurs naturally in many plants and resins. It is also used pharmacologically as an anticarcinogenic agent that reduces the frequency or rate of spontaneous or induced tumors independent of the mechanism involved. Caffeoyl-shikimic is acidic and has a molecular weight of 336.29344 [g/Mol], and like many other phenolic compounds, it serves multiple functions including acting as anti-oxidants and signaling molecules. It is also used in the pharmaceutical industry as it displays anti-tumor properties [41].

Anti-Oxidant Properties

Many studies that have been conducted in the recent past have shown that diets containing high amounts of phytochemicals can provide protection against prooxidant-induced diseases. This is as a result of photochemical high antioxidant activities. Antioxidants preclude prooxidant induced tissue damage by preventing the formation of these prooxidants, by scavenging for the prooxidants or promoting their decomposition. Antioxidants have also been found to be caloric restriction mimetic, the only intervention known to lengthen the median lifespan of animals [44]. OPP has been shown to display antioxidant properties and confer positive outcomes on degenerative diseases in various animal models without evidence of causing toxicity. Whether in healthy or diseased states, the utilization of OPP in the nutraceutical market is subject to understanding the molecular mechanisms involved in bringing about the positive benefits observed [45]. A study was conducted to identify the in vivo biological effects and molecular mechanisms of these compounds during healthy states in three major organs (heart, liver and spleen) of the mouse on a normal diet, via microarray gene expression profiling.

It was found that OPP had great potential in scavenging for the prooxidants and causing them to decompose, hence reducing the toxicity of oxygen compounds in the body [2]. Besides the water-soluble phenolic acid-rich complex, the oil palm (Elaeis guineensis) also contains an excellent repertoire of antioxidant phytochemicals, many of which are lipid soluble antioxidants, such as carotenoids (vitamin A precursors), as well as tocotrienols and tocopherols (vitamin E isomers) [46].

Anticancer Properties

Cancer occurs as a consequence of the accumulation of mutations in genes whose protein products are important in the control of cell proliferation, differentiation, and apoptosis. Normally, before a person develops a tumor, several cancer-promoting factors have to occur and with a few exceptions, one mutation is not sufficient. The main mechanisms for the development of cancer are; (a) impairment of a DNA repair pathway, (b) the mutation of a tumor suppressor gene, (c) the transformation of a normal gene (the proto-oncogene) into an oncogene or (d) mutations in genes involved in apoptotic control [47].

In a study carried out to assess the anticancer properties of oil palm phenolics, human breast and prostate cancer cells were cultured in vitro either in the presence and absence of various doses (weight %) of palm phenolics. It was noted that the palm phenolics inhibited the growth of human breast and prostate cancer cells in a dose-dependent manner whereas cancer cells that were not supplemented with palm phenolics continued to proliferate and grow [48].

Another study that used animal models, (rats), Dimethyl hydrazine (DMH) was used as the chemical trigger, to induce colon carcinogenesis in the rats through multiple doses of DMH intra-gastrically. The drinking water of the experimental group of animals was supplemented with palm phenolics, whereas the control group of animals was provided with water and not supplemented

with any palm phenolics. All the experimental rats were maintained on normal rat chow for the duration of the study. Rats that had their water supplemented with the palm oil phenolics demonstrated significantly lower colon polyps and tumors compared to the control animals that were not supplemented with them [49].

Effect on Apoptotic Pathways

An important mechanism of cytotoxicity in the prevention and treatment of cancer is the apoptosis or programmed cell death pathway. Apoptosis is characterized by several morphological and biochemical changes including blebbing of the plasma membrane, condensation of cytoplasm and nucleus, as well as degradation of DNA. The inability of cells to undergo apoptosis is a key factor in the pathogenesis of malignancies [50].

Recently, there has been increasing interest in the use of palm oil and other oil palm components in tumor inhibition and regression. In this study, the authors determined the effects of water-soluble OPP on tumor and normal cell lines, and further checked for the mechanism of cell death caused by OPP in cell lines which were inhibited by the extract. They also determined the in vivo effects of OPP on J558 myeloma tumor growth in syngeneic BALB/c mice [51]. Apoptotic cells were detected using a calorimetric detection system in all tumor cells treated with OPP. There was an increase in the number of apoptotic cells in groups incubated with higher concentrations of OPP. No apoptotic cells were detected in the normal and untreated cells and this was found to be consistent with the results of the DNA fragmentation assay [52]. Isolation and concentration of the phenolics from this palm oil result in OPP. In vitro exposure of tumor cells to various concentrations of OPP revealed there was a dose-dependent growth arrest and cell death effects of the extract. In the dose experiments, when estrogen receptor positive human breast adenocarcinoma (MCF7), human lung carcinoma (A549) and mouse IgA-secreting myeloma

(J558) cell lines were incubated with different doses of OPP. The extract showed a distinct inhibitory effect on the viability and proliferation of the cells at all doses [53]. These findings are concurrent with the studies on palm oil vitamin E (tocotrienol-rich fraction) tested on human breast cancer cells, where similar inhibitory actions were reported. The accelerated decrease in the viability of the carcinoma cells started after 24 hours of treatment with the extract and was highest at the end of the incubation periods. This effect was more pronounced with higher concentrations of OPP [45]. In contrast, OPP was shown to enhance the growth and proliferation of normal vertebrate and invertebrate cell lines in a dose-dependent manner. Enumerated by the MTT assay, OPP doses below 2000 µg/ml were found to enhance the growth of the C6/36, BHK, and MDCK cell lines. However, cell viability decreased dramatically after day 7. This could be due to lack of nutrients or space for cell growth [49].

In general, cell death can occur by either necrosis or apoptosis (programmed cell death). To determine the cell death mechanism of the tumor cells, two methods of detecting DNA fragmentation were used. Nuclear fragmentation was detected in all samples of tumor cells treated with various concentrations of OPP, indicating that OPP had induced apoptosis in these cells in vitro [52]. These results were further confirmed using the TUNEL assay, which showed that the intensity of cells undergoing apoptosis was greater with higher concentrations of OPP used. The selective induction of cell death in tumor cells could be due to the presence of gallic acid in OPP. Agrawal in another study also showed that the tocotrienol-rich fraction of palm oil activated on p53, resulting in DNA fragmentation and ultimately apoptosis [54].

Effect on Inflammatory Pathways

12-O-tetradecanoyl-phorbol-13-acetate (TPA) is diester of phorbol and a potent tumor promoter often employed in biomedical research to activate the signal transduction enzyme protein

kinase C (PKC). TPA effects on PKC result from its similarity to one of the natural activators of classic PKC isoforms, diacylglycerol. In animal models, palm oil has been shown to significantly protect against 12-O-tetradecanoyl-phorbol-13- acetate-mediated skin tumorigenesis in Swiss albino mice [44]. Using Illumina microarrays, it was found that the atherogenic diet-induced inflammation, oxidative stress and increased metabolite turnover of cells in the spleen, liver, and heart. On the other hand, OPP showed signs of attenuating these effects. It increased the response of unfolded proteins in the liver, attenuated antigen presentation and processing in the spleen and upregulated antioxidant genes in the heart. Current quantitative reverse transcription-polymerase chain reactions validated then the fold changes observed in the microarray gene. While serum cytokine profiling showed that OPP attenuated inflammation by modulating the Th1/Th2 axis toward the latter [37].

The most invasive cancers normally produce the broadest spectrum and the highest levels of chemokines. An important example of a cytokine receptor pair involved in cancer invasion and metastasis is Cxcl12/Cxcr4. In vitro, the CXCL12 ligand stimulated breast cancer cells to carry out invasive, including a pseudopodial protrusion, directed migration, and penetration of extracellular matrix barriers. In vivo, metastasis to CXCL12-rich lung tissue was blocked in animal models by treatment with a neutralizing anti-human CXCR4 monoclonal antibody. Cxcl12 is an important mediator of advanced cancer and contributes significantly to the lethal phenotype. In the present study, the down-regulation of Cxcl12 in tumors was observed in OPP treated mice resulting in a reduction in invasiveness and metastatic potential of these tumors [40].

Interleukin-12 (IL-12) is a heterodimeric cytokine produced mostly by phagocytic cells in response to foreign bodies, and to some degree by B lymphocytes. IL-12 induces cytokine production, primarily of IFN-gamma, from NK and T cells. It acts as a growth factor for activated

NK and T cells, enhancing the cytotoxic activity of NK cells, and favors cytotoxic T lymphocyte generation. It is demonstrated that OPP causes a significant decrease in proinflammatory interleukin [IL-12] and a significant increase in anti-inflammatory interleukin-13, resulting in control of inflammation [48].

Effect on Epigenetic Control

Furthermore, OPP down-regulated genes that are usually overexpressed and associated with cancer proliferation and invasion. These genes include signal transducer and activator of transcription 3 (Stat3), chemokine (C–X–C motif) and ligand 12 (Cxcl12). The transformation of various primary cancer and tumor-derived cell lines requires oncogenic Stat3 signaling, and is dependent on this activated protein, thus, when these cell lines are treated with antisense or dominant negative constructs directed at Stat3, they undergo growth arrest or even apoptosis [53].

According to Niu, blocking Stat3 in cancer cells up-regulates the expression of p53, leading to p53-mediated tumor cell apoptosis. In addition, inhibition of constitutive Stat3 activity in hematopoietic malignancies such as non-Hodgkin's lymphoma and multiple myeloma sensitizes these resistant cancers to chemotherapeutic drug-mediated apoptosis and also suppresses the growth of prostate and astrocytoma cancer cells [55]. Resveratrol, which has been reported to have anti-tumor properties, also inhibit Stat3 signaling in transforming mouse fibroblasts as well as human prostate, pancreatic and breast carcinoma cell lines [53].

Effect on Cell Cycle

Functional analysis carried out using microarray data obtained from tumors of mice indicated that genes involved in the cell cycle were differentially expressed in the OPP -treated group. Genes such as cyclin A2 (Ccna2), cyclin B1(Ccnb1), polo-like kinase 1(Plk1), mitotic arrest deficient 2, homologue-like 1 (Mad2l1), cell division cycle 20 homologue (Cdc20), histone

deacetylase 6 (Hdac6) and minichromosome maintenance deficient 6 (Mcm6) were down-regulated by OPP. On the other hand, genes such as cyclin D2 (Ccnd2), cyclin E2 (Ccne2), cyclin-dependent kinase 4 (Cdk4) and cyclin-dependent kinase 2 (Cdk2) were up-regulated by OPP [43].

In Ccne2 and Ccna2 genes, obtained from real-time qRT-PCR, the directions and fold changes for two genes was comparable with those obtained using the microarray technique, thus validating the microarray results obtained. The regulation of cell-cycle genes found in the present study suggests that OPP may inhibit tumor growth in vivo by inducing a G1/S phase arrest in the cell cycle. This is consistent with observations made in several cancer cell lines, in which cell cycle arrest was brought about by other phenolic antioxidants such as phenolic acids, hydroxytyrosol, resveratrol, piceatannol, genistein and luteolin [56].

The opposite direction of regulation of these genes by OPP indicates that OPP may help sensitize tumor cells towards chemotherapy, and this might be vital when used together with selected chemotherapeutic agents for enhanced cytotoxicity in cancer cells [36].

Summary

The burden of chronic diseases is rapidly increasing worldwide. Oxidative stress is a unifying mechanism in the etiology of many chronic diseases, including cancer. As such, dietary antioxidants such as widespread plant phenolics may be important for the prevention and treatment of chronic diseases. The discovery that the aqueous stream of the oil palm milling process contains potent phenolics has raised the possibility that a major disposal liability can be turned into beneficial use against chronic disease processes [47].

The use of antioxidant -phenolic-rich palm oil with potent radical scavenging activity, may play a vital role in the prevention and treatment of many chronic illnesses that result from prooxidants' cell damage. It is reported that palm oil has the capacity to modulate the effect of

oxidative stress on serum as well as antioxidant enzymes in membranes of the liver and kidneys. It also suppresses pre-neoplastic mammary epithelial cell proliferation, inhibits diesel-exhaust induced lung tumorigenesis, tumor promotion, and modulates the immune system [57].

As the transformation of normal cells to malignant cells is brought about by, spontaneous mutation during daily cell division or may be induced by: chemical carcinogens, physical carcinogens, and viruses. If these two factors can be prevented or be inhibited, then it will be possible to use oil palm phenolics to greatly reduce the incidence of cancer and other chronic diseases [40]. Experiments have been carried out and have shown that cancer being as a result of uncontrolled growth and spread of abnormal cells, the regulation of cell-cycle genes inhibits tumor growth. Moreover, the prevention of inflammatory pathways can be protective. Since OPP has demonstrated that it possesses many anti-cancer properties, it may be considered as a viable alternative in the fight against cancer [37].

Previously our lab investigated the in-vitro effects of OPP in PANC-1 and BxPC-3 pancreatic cancer cell lines. The study showed a therapeutic potential of OPP against pancreatic cancer in both cell lines. OPP inhibited cell growth, proliferation (Fig. 1-1), migration and invasion of cell lines PANC-1 and BxPC-3 [83]. Furthermore, the study also explored the molecular pathways through which apoptosis was induced. The results demonstrated a down-regulation of NF-kB pathway a dose-dependent apoptosis induction (X. Ji, et al, 2015) [83]. Published results from our laboratory further demonstrate OPP's ability in treating pancreatic cancer. However, because OPP contains a very large number of compounds, it is important to evaluate the subfractions of OPP responsible for its therapeutic effects. Thus, my project aims to investigate the effect of subfractions of OPP in pancreatic cancer cell lines. This will help to identify the active subfraction and possible the compounds contained in it.

Table. 1-1 Stages of Pancreatic Cancer.

AJCC stage	Stage grouping	Stage description
0	Tis	Cancer is confined to the top layers of pancreatic duct cells and has not invaded internal tissues.
	No	Has not spread to lymph nodes.
	MO	No distant metastases.
IA	T1	Cancer is confined to the pancreas and is about 2 cm.
	N0	Has not spread to lymph nodes.
	M0	No distant metastases.
IB	T2	Cancer is confined to the pancreas and is larger than 2cm and not more than 4cm.
	N0	Has not spread to lymph nodes.
	M0	No distant metastases.
IIA	T3	Cancer is confined to the pancreas but is bigger than 4cm.
	N0	Has not spread to lymph nodes.
	M0	No distant metastases.
IIB	T1	Cancer is confined to the pancreas, smaller than 2cm across T1.
	N1	Has spread to not more than 3 nearby lymph nodes.
	M0	No distant metastases.

	T2	Cancer is confined to the pancreas, is larger than 2cm but smaller than 4 cm
	N1	Has spread to no more than 3 nearby lymph nodes.
	M0	No distant metastases.
	T3	Cancer is confined to the pancreas and is larger than 4cm across T3.
	N1	Has spread to no more than 3 nearby lymph nodes.
	M0	No distant metastases.
III	T1	Cancer is confined to the pancreas, smaller than 2cm across T1.
	N2	Has spread to 4 or more lymph nodes.
	M0	No distant metastases.
	T2	Cancer is confined to the pancreas, is larger than 2cm but smaller than 4cm
	N2	Has spread to 4 or more lymph nodes.
	M0	No distant metastases.
	T3	Cancer is confined to the pancreas and is larger than 4cm across T3.
	N2	Has spread to 4 or more lymph nodes.
	M0	No distant metastases.
	T4	Cancer cells are growing outside the pancreas and into nearby major blood vessels.
	Any N	May or may not have spread to nearby lymph nodes.

	M0	No distant metastases.
IV	Any T	Cancer tumor can be any size.
	Any N	May or may not have spread to nearby lymph nodes.
	M1	Distant metastases
TX		The primary tumor is inaccessible
T0		No evidence of a primary tumor.
NX		Regional lymph nodes are inaccessible.

(Dragovich T., et al, Pancreatic Cancer Staging, Medscape, 2017)

Figure. 1-1. Anti-proliferative effect (MTS assay) of OPP on PANC-1 and BxPC-3.
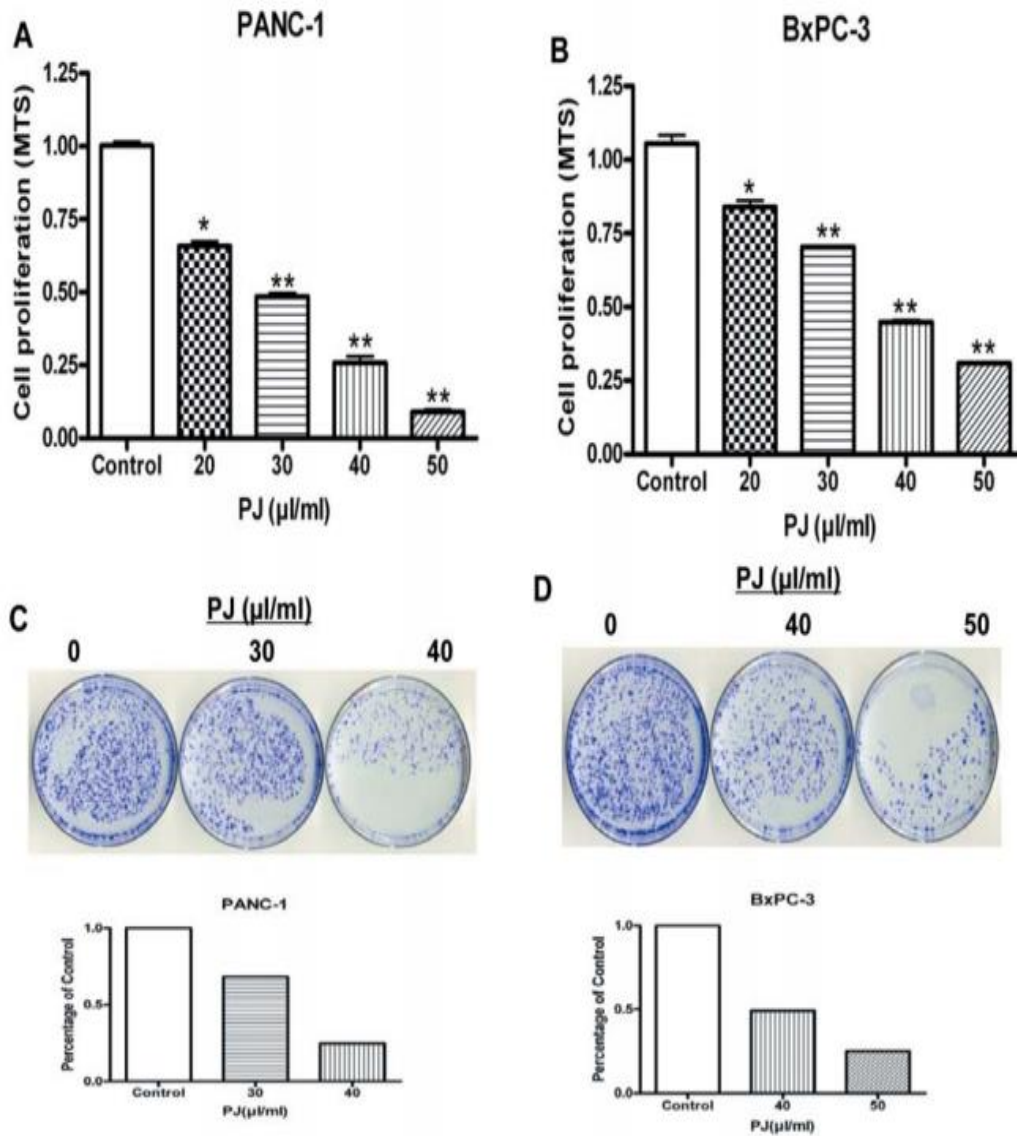
Effect of OPP on pancreatic cancer cell lines PANC-1 and BxPC-3 regarding survival and growth (A and B) and clonogenicity (C and D). Results are presented as mean ± SEM of three assay replicates. *p<0.05 and **p<0.01 versus respective DMSO-treated controls (0 μl OPP). The number of cells counted in the control treatment was considered 100% and the number of cells in OPP-treated cells was calculated relative to this control [83].

(X. Ji, et al, Anticancer Research, 2015) [83]

CHAPTER 2: Hypothesis

The overall goal of this study is to evaluate and establish the anti-cancer properties of OPP (oil palm phenolics) against different cancer types. Our laboratory has been investigating this with positive results in pancreatic cancer. However, to establish it as a therapeutic agent, the active fractions of OPP need evaluation. To achieve this goal, we hypothesized that one or more subfractions of OPP will be effective in reducing the growth and proliferation of pancreatic cancer cells (paca).

Specific Aims

To test the hypothesis the following specific aims are proposed:

Aim 1: To evaluate the effects of oil palm phenolics (OPP) subfractions on the growth of pancreatic cancer cells. The active subfraction will be identified and investigated for its effect on; cell proliferation, inhibition, cell invasion, and migration.

Aim 2: To evaluate the identified active OPP subfraction for parameters involved in apoptosis including gene expression of mTOR, and BCL-2.

MATERIAL AND METHODS

Materials, Reagents, and Methods

The MTS assay was carried out in two parts to determine both the potent OPP fraction and its effect against Panc1 and BxPC-3 PACA cancer cell lines. The data from the assays was confirmed from colony forming assays (clonogenic assays). Similarly, cell invasion and migration capabilities after the administration of OPP were examined in the wound healing and cell invasion assays. The molecular mechanisms through which cell invasion and migration were explored through Real-Time PCR by testing the expression of MTOR, BCL-2 downstream genes at the mRNA and protein levels.

OPP was fractionated into subfractions based on solubility/extractability in different solvent systems. The freeze-dried fractions (n=13) were provided to us by MPOB, Kuala Lumpur, Malaysia. Bovine serum albumin (BSA) was purchased from Sigma- Aldrich (St. Louis, Mo., USA), DMSO, TNF- α, the protein assay kit was obtained from Bio-Rad Labs (Hercules, Calif., USA). PACA cell lines of human BXPC-3 and Panc1 were obtained from ATCC, RPMI medium obtained from Mediatech, Manassas, VA was used as a growth medium for the cells, and β- actin, BCL-2 and mTOR antibodies were obtained from Cell Signaling Technology, Denvers, MA. In this study, we evaluated the fractions of OPP to find out the active one against PACA cells. The MTS assay was carried out to evaluate the most active fraction of OPP against Panc-1 cell line.

Cell Culture and Treatments

The treatment medium was prepared by mixing OPP (<0.1% DMSO as a vector) in the RPMI medium whereas the control cells were only treated with RPMI media that contained <0.1% DMSO. The RPMI medium was supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin in 5% CO^2 at 37°C and the PANC-1 and BxPC-3 pancreatic cell lines were first cultured in this medium. The cell culture medium was renewed every 2-3 day. Cells that had adhered to each other were detached through incubation with trypsin- EDTA and centrifuged at 1500 RPM. OPP concentrations of 0, 0.4 µM, 0.6 µM, 0.8 µM and 1 µM that had <01% DMSO were used as the treatment concentrations.

Cell Viability Assay

The antiproliferative effects of subfractions of OPP on the pancreatic cancer cell lines were evaluated through the MTS assay. 5×10^5 of both PANC1 and BXPC-3 cells were seeded in a 96 well- plate and incubated overnight, after which the cells were treated with fresh medium containing <0.1% DMSO and treatment medium with of 0, 0.4 µM, 0.6 µM, 0.8 µM of OPP. After

72 hours of treatment, 20 µl of cell Titer 96 aqueous one solution reagent from Promega was added to each well, and they were incubated for 2 hours at 37°C in a humidified 5% CO_2 atmosphere. The absorbance of the cells was then measured with the Bio- Tek EL × 800 plate reader at 490nm. In each variant of the experiment, six replicates were carried out to minimize errors. This was used to identify the active subfraction of OPP. The remaining assays were conducted using the active subfraction of OPP.

Cell Invasion Assay

BD Biocoat invasion kit (BD, San Jose, CA) was used to determine the ability of the pancreatic cancer cell lines, PANC-1 and BxPC-3 to pass filters that are coated with Matrigel. In the assessment, both cell lines were extracted in an aqueous environment with PBS before they were re-suspended into each upper chamber of six-well plates. These well plates had a serum-free medium either in the presence or absence of treatment (0, 0.2 µM, 0.6 µM, 0.8 µM). 3ml of the culture medium with 15% FBS was then added to the lower chambers of the six-well- plates and incubated for 20 hours. The cells in the upper chamber were then removed with the use of a cotton swab, and those that invaded across the Matrigel to the lower membrane surface were fixed with 4% Paraformaldehyde before they were stained with 2% crystal violet.

The cells that invaded the lower surface of the membrane were then counted and pictures were taken with a camera that was attached to a light microscope and data was presented from cells that were adhered to the lower surface from randomly chosen fields. Cell Invasion Assay experiments were performed in triplicate, and a similar procedure was followed until the cells were incubated for 20 hours in a BD Biocoat invasion kit. After the incubation, the cells in the upper chamber were removed with the use of a cotton swab and 50 µl of the MTS reagent was added to the lower chamber. The absorbance of the media in the lower chamber was then measured at

490nm with the use of Bio- Tek EL × 800 plate reader. These experiments were also carried out in triplicates.

Cell Migration Assay

The cell migration and wound healing assays were used to indicate the migration speed of the cells. In the assay, PANC-1 and BxPC-3 cell lines were seeded in a 6-well- plate in the concentration of 1×10^6 cells per well, and they were incubated for 36 hours. The culture media was then removed and treated with or without the treatment media. A 200 µl pipette tip was used to make scratches on the cells, and these were photographed immediately and incubated for 30 hours. The wound areas were then washed with PBS thrice to make certain that loosely attached cells were not held. The plate was then imaged, and the width of the scratch was measured with a Nikon H 600L that was connected to a microscope.

Real-time Quantitative Polymerase Chain Reaction

One million BxPC-3 and PANC-1 cells were seeded in 100mm dish per plate, and they were incubated for 24 hours. After the incubation, the culture media was replaced with treatment or control medium, and they were incubated for another 72 hours. The isolation of total RNA was then carried out with the use of RNeasy Mini Kit from QIAGEN according to the manufacturer's protocols. 1000ng of total RNA obtained from each sample was exposed to the synthesis of the first complementary DNA strand through the use of high capacity RNA to cDNA master mix (Applied Biosystems, Foster City, CA) in a total volume of 50ml.

Quantitative RT- PCR was then carried out as part of the analysis of gene expression. For PCR amplification, the appropriate primers of MMP primer sequence was used. Each PCR reaction was performed in Eppendorf master cycler realplex 4 at 25°C for 10 minutes, and diluted cDNA (2 µL) and 2 µL of each reverse and forward primer and 12.5 µL master mix were used.

These were then incubated at 48°C for 30 minutes and 95°C for 5 minutes. The expression values were normalized with the Bactin antibiotic. The gene expression analysis tests were carried out in duplicates.

NF- ƙß Binding Activity

This step determined the NF- ƙß transcription factor binding activity. The PANC1 and BXPC-3 cell lines were seeded in Petri dishes and incubated for 24 hours. The cells were then treated with varying concentrations with or without OPP for 72 hours (0, 0.5 µM, 0.75 µM, 1 µM) before they were collected and washed with the PBS. Manufacturer protocols were then used to extract the nuclear protein with the NE- PER Nuclear and Cytoplasmic extraction reagent extraction kit (Thermo Scientific, USA). Concentrations of the nuclear protein were then determined with the use of the Pierce BCA protein assay kit and the NF- ƙß DNA binding ability was determined with the use of the NF- ƙß Filter Plate Assay Kit from Signosis according to the protocols described by the manufacturer. An NF-ƙß DNA binding complex was made through the mixing of the biotin-labeled DNA sequence of NF- ƙß and 3µg nuclear extract. After the complex was made, 10 µL TF binding buffer mix was added along with 2 µL NF-ƙß probe 3 µg and distilled water to each sample and these were brought to a volume of 20 µL. the unbound NF-ƙß probe was filtered out while the filter plate was used to retain the bound NF- ƙß probe. The bound NF- ƙß probe was labeled and eluted from the filter, collected and transferred to a hybridization plate for quantitative analysis. Further, the NF- ƙß probe was detected with the use of streptavidin- HRP. The Ultra Multifunctional Microplate Reader from Tecan Vienna Virginia was used to measure the luminescence of the probe.

CHAPTER 3: RESULTS

MTS Assay on OPP fractions

The MTS assay was performed to evaluate the freeze-dried fractions provided by MPOB. Of these data, it was shown that subfraction-5, 8, 10 were more active against paca cell lines. However, fraction 5 was identified as being the most potent (Fig. 3-1). Remaining experiments were performed using this fraction.

Anti-proliferative effect of OPP fraction 5 using MTS Assay

The MTS assay showed a dose-dependent decrease in cell growth and proliferation in both Panc1 and BxPC-3 cells with the addition of OPP subfraction-5. After 72 hours of incubations with 0, 0.4, 0.6, 0.8, 1 µM of OPP-subfraction 5 doses, ~ 10, 40, 80 and 85% of cell growth inhibition was observed, respectively, compared to control in Panc1 cells (Fig. 3-2). Similarly, the BxPC-3 (Fig. 3-3) cell line showed ~ 25, 20, 55 and 87% cell growth inhibition, respectively, with the same doses and conditions.

Inhibition of cell invasion and migration by OPP.

We observed a statistically significant, dose-dependent (0.2, 0.6, 0.8 µg/mL) decrease in cell invasion with OPP-subfraction 5 treatment in Panc-1 cell lines (Fig. 3-4). In BxPC-3 cell lines, we observed a significant, dose-dependent (0.6, 0.8 µg/mL) decrease in cell invasion in Matri gel invasion assay (Fig. 3-5). In Panc1, we observed that a low number of cells migrated through the membrane as compared with untreated cells (Fig. 3-4) (P <0.05)

Wound Healing Assay

The wound healing assay was performed to investigate the migration of Panc-1 (Fig. 3-6) and BxPC-3 (Fig. 3-7) after OPP treatment. OPP significantly inhibited the migration of cells after 30h treatment with 0 and 0.8 ug/mL.

Inhibition of NF-κB DNA-binding activity with OPP. We investigated whether OPP modulates the nuclear translocation of NF-KB. Based on the results of the NF-KB filter plate, OPP subfraction-5 significantly inhibited the DNA-binding activity in a dose-dependent manner in both PANCL and BxPC-3 cell lines tested (Fig. 3-8).

Real-Time Quantitative PCR for Gene Expression Analysis

To test the effect of OPP subfraction-5 on, MTOR and BCL2 at the gene level. Real-Time PCR was performed to measure the mRNA expression of BCL2 in both PANC-1 (Fig. 3-9) and BxPC-3 (Fig. 3-10) cell lines followed with mTOR expression for PANC-1 (Fig. 3-11) and BxPC-3 (Fig. 3-12). Control was tested against the treatments 0.2, 0.4 and 0.6. A dose-dependent reduction in the BCL2 gene expression was observed as shown below.

Figure 3-1. MTS Assay OPP fractions 1, 4, 5, 8, 10, 13.
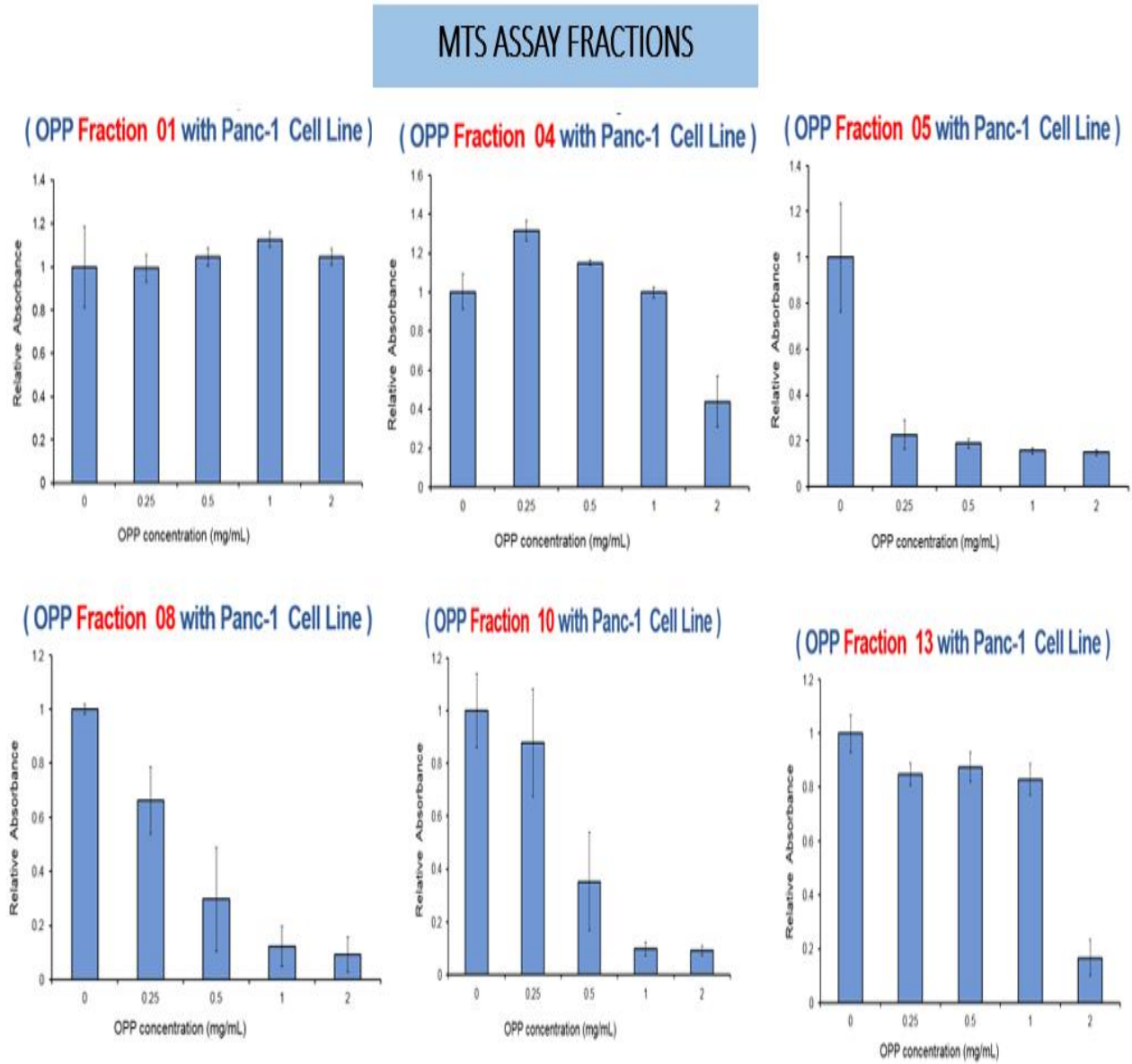
Results showed PANC-1 cell were more sensitive to OPP fraction-5 when compared fractions 1, 4, 8, 10 and 13.
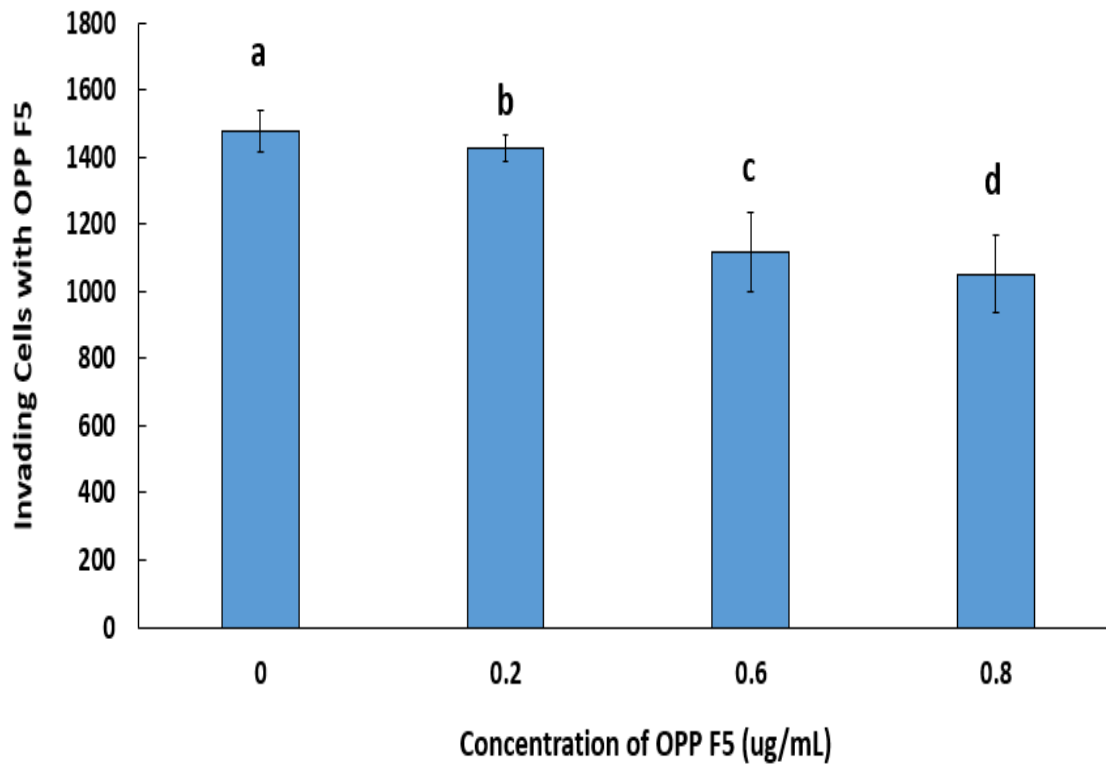
Figure 3-2. Cell Proliferation upon addition of OPP Fraction-5 PANC-1 Cell Line
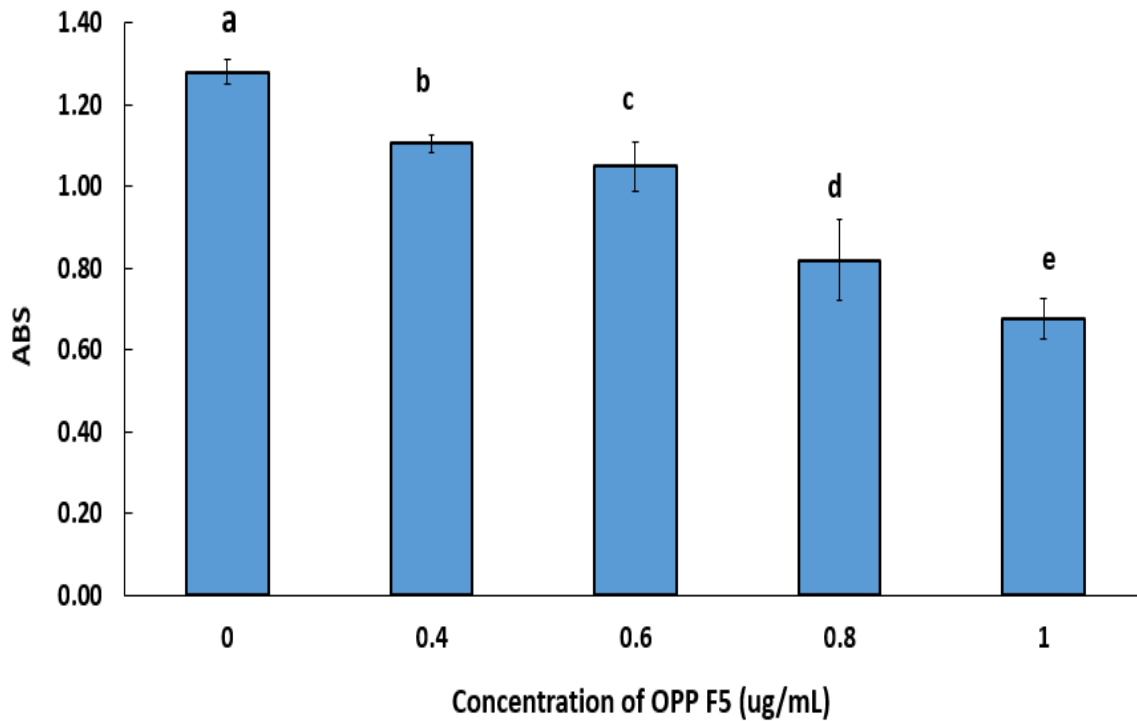
Data is represented as mean ± S.E.M. Lowercase manuscript letters [a, b, c] **P < 0.05**, represents differences between the treatments in relation to control (0). And analysis was conducted using Paired and Independent t-test comparison, and One-way ANOVA.

Figure 3-3. MTS assay upon addition of OPP fraction - 5 Cell Proliferation for BxPC-3 Cell Line
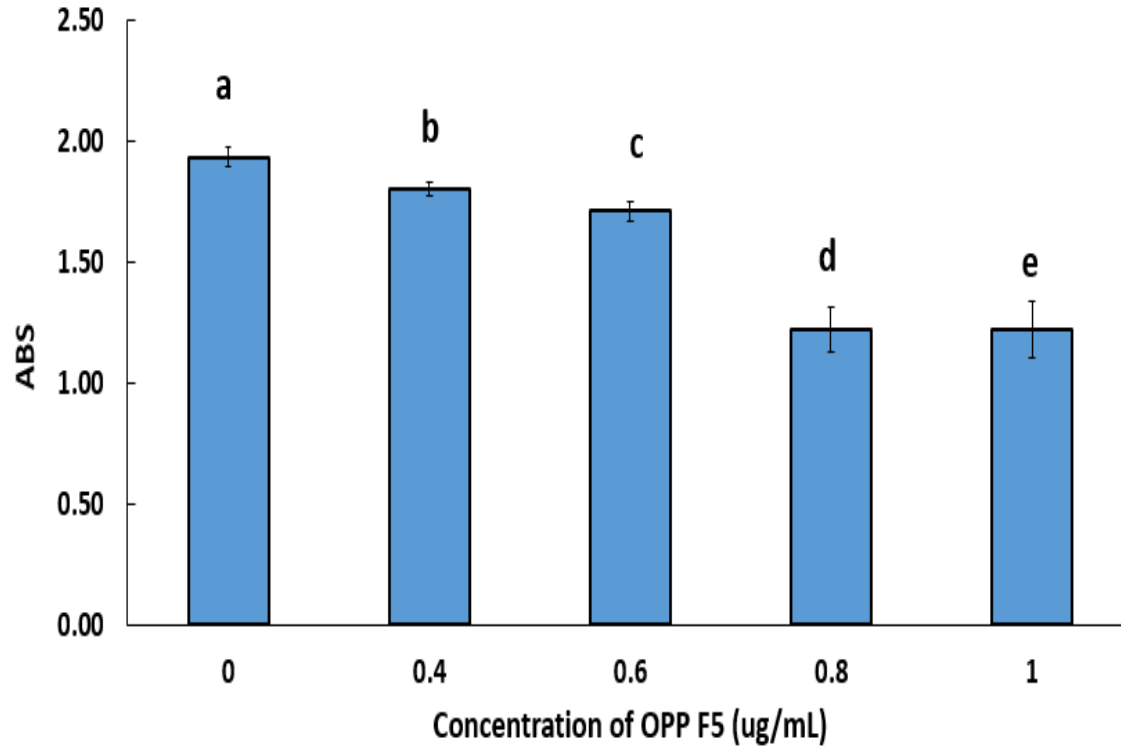

Data is represented as mean ± S.E.M. Lowercase manuscript letters [a, b, c] $P < 0.05$, represents differences between the treatments in relation to control (0). And analysis was conducted using Paired and Independent t-test comparison, and One-way ANOVA.

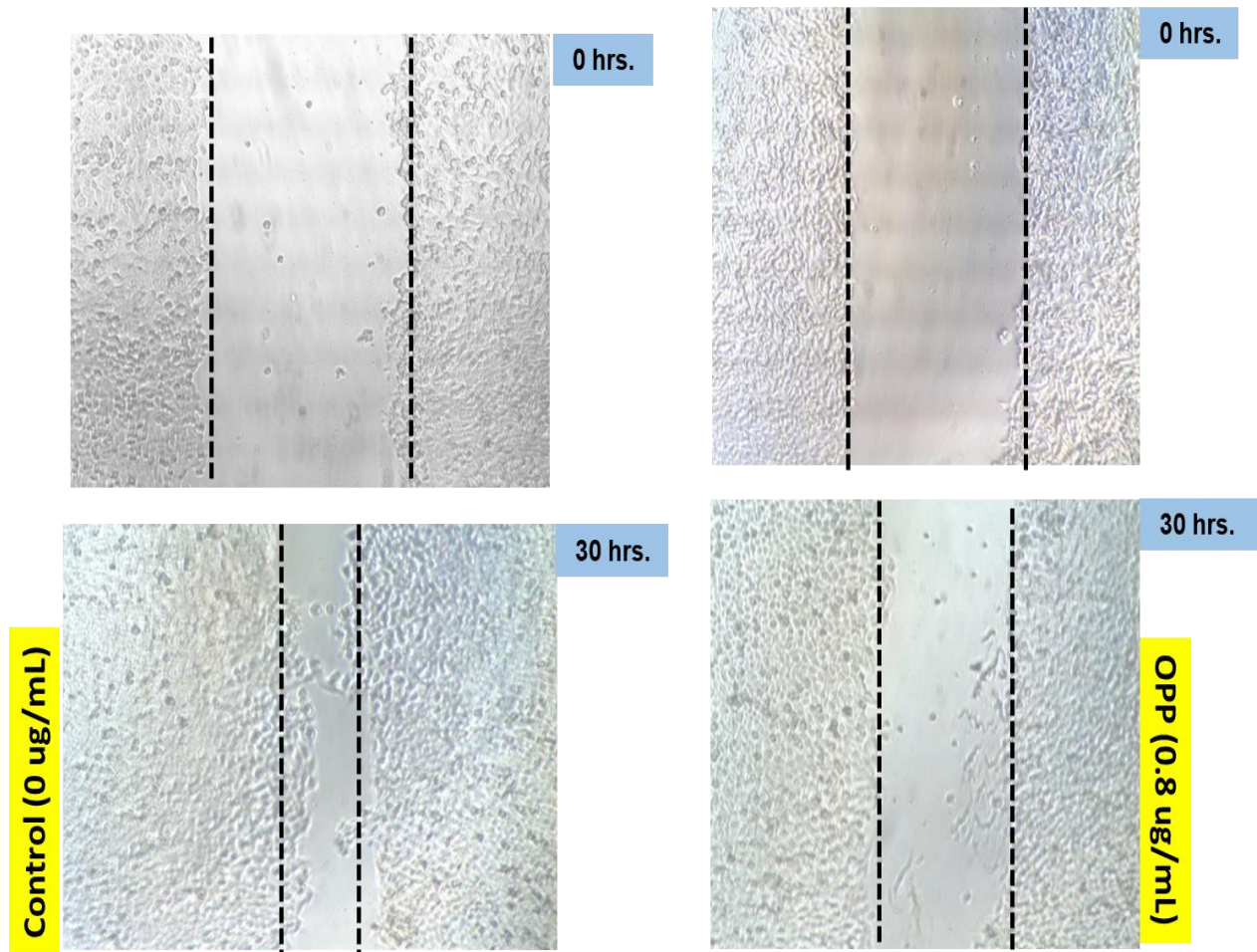**Figure 3-4**. Cell Invasion upon adding OPP fraction-5 for Cell Line PANC-1

Data is represented as mean ± S.E.M. Lowercase manuscript letters [a, b, c] **P < 0.05**, represents differences between the treatments in relation to control (0), and analysis was conducted using Independent t-test comparison, and One-way ANOVA.
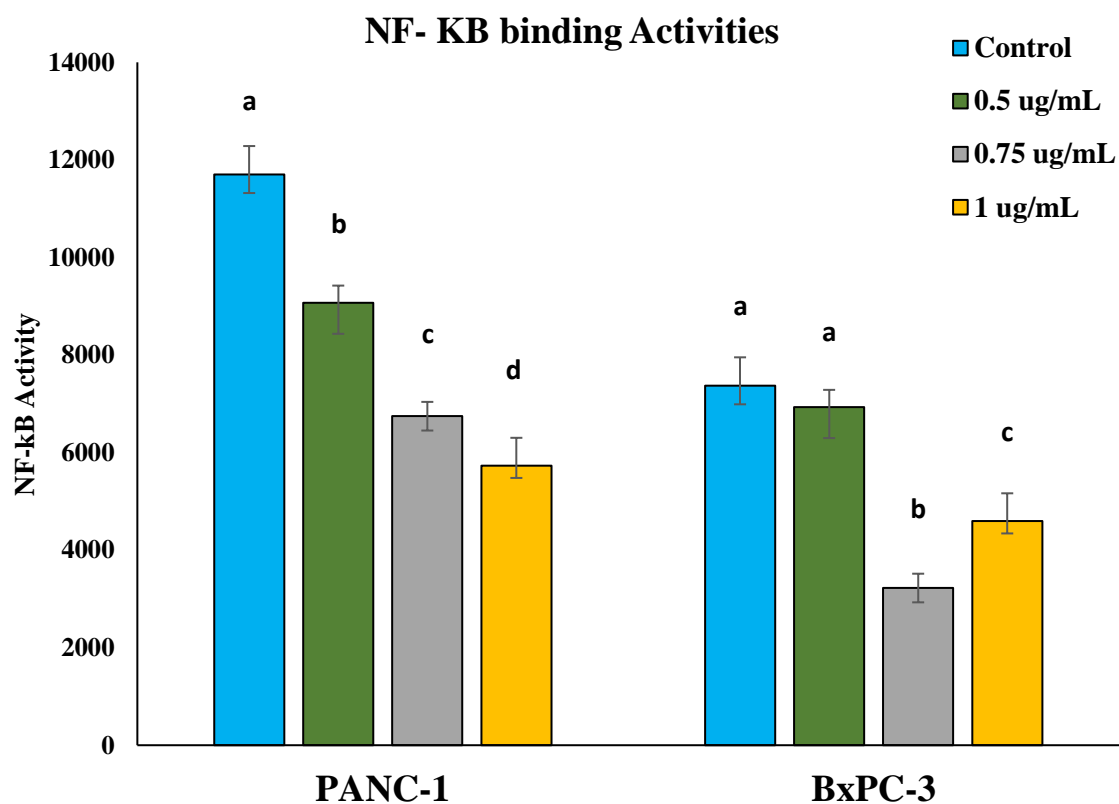
Figure 3-5. Cell Invasion for Cell Line BxPC-3
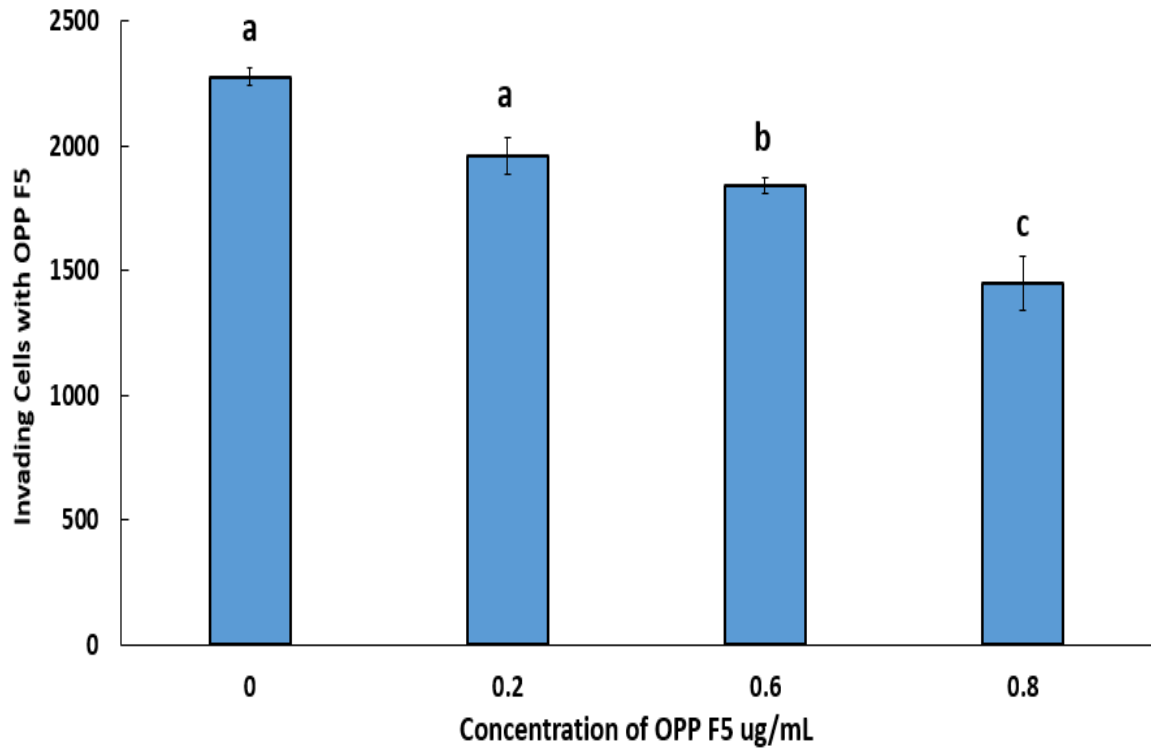
Data is represented as mean ± S.E.M. Lowercase manuscript letters [a, b, c] **P < 0.05**, represents differences between the treatments in relation to control (0). The analysis was conducted using Independent t-test comparison, and One-way ANOVA.

Figure 3-6. Wound Healing Assay for Panc-1
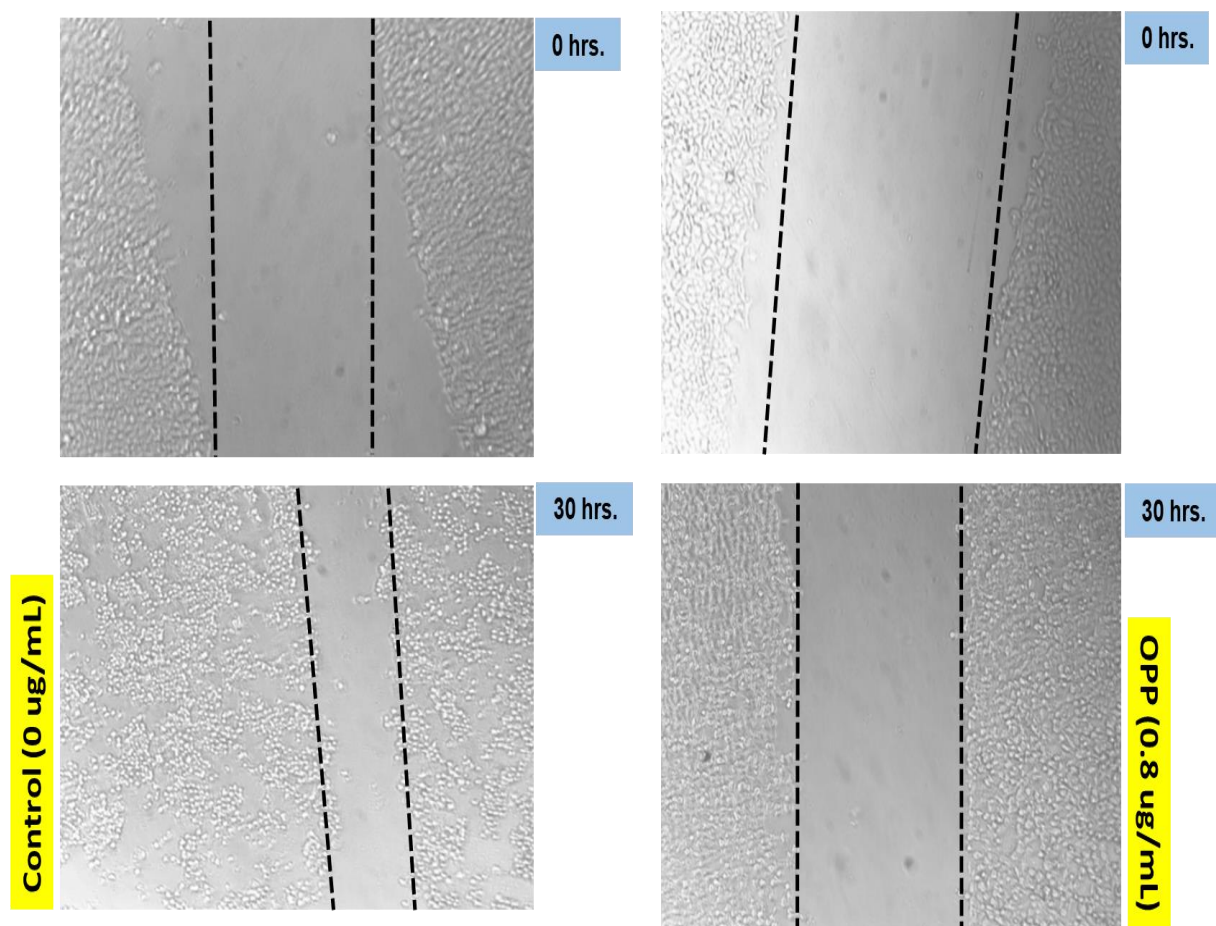
The wound healing assay indicated a Dose-dependent inhibition of PANC-1, and migration because of OPP treatment. Pictures above are shown at, 1ug/mL. Uniform wounds were created by scratching in confluent cultures that were treated with OPP for 30 hours. A microscope was used at the 10× objective to capture wound healing images after 30 hours.

Figure 3-7. Wound Healing Assay for BxPC-3

The wound healing assay indicated a Dose-dependent inhibition of BxPC-3, and migration because of OPP treatment. Pictures above are shown at, 1ug/mL. Uniform wounds were created by scratching in confluent cultures that were treated with OPP for 30 hours. A microscope was used at the 10× objective to capture wound healing images after 30 hours.

Figure 3-8. NF-kB Binding Activity for PANC-1 and BxPC-3.

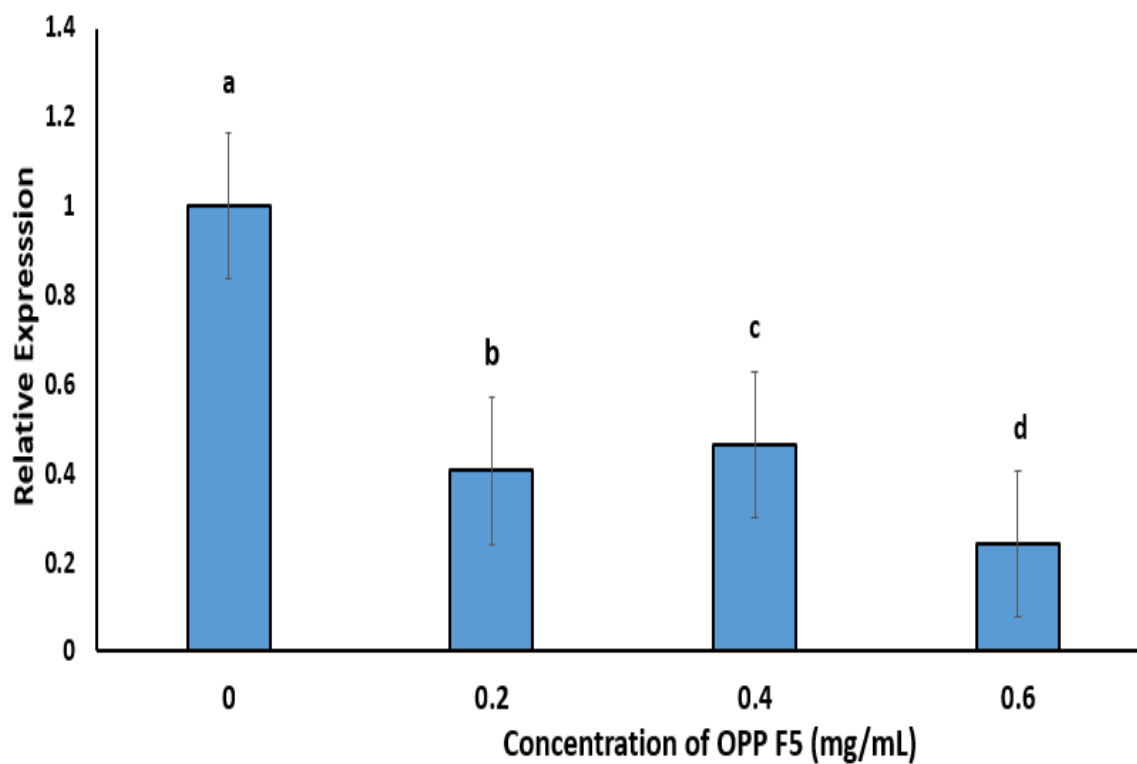
Data is represented as mean ± S.E.M. Lowercase manuscript letters [a, b, c] **P < 0.05**, represents differences between the treatments in relation to control (0), and analysis was conducted using Independent t-test comparison, and One-way ANOVA.

Figure 3-9. BCL-2 Expression: PANC-1 Cell Line
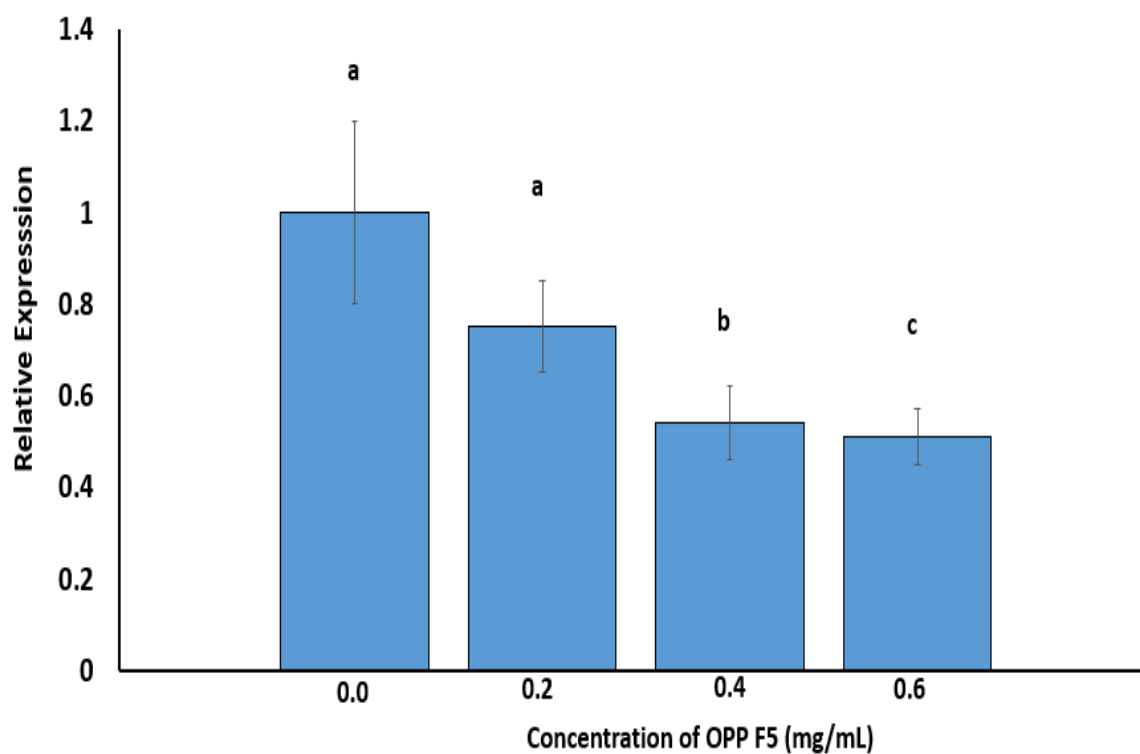
Data is represented as mean ± S.E.M. Lowercase manuscript letters [a, b, c] **P < 0.05**, represents differences between the treatments in relation to control (0), and analysis was conducted using Independent t-test comparison, and One-way ANOVA.

Figure 3-10. BCL-2 Expression: BxPC-3 Cell Line
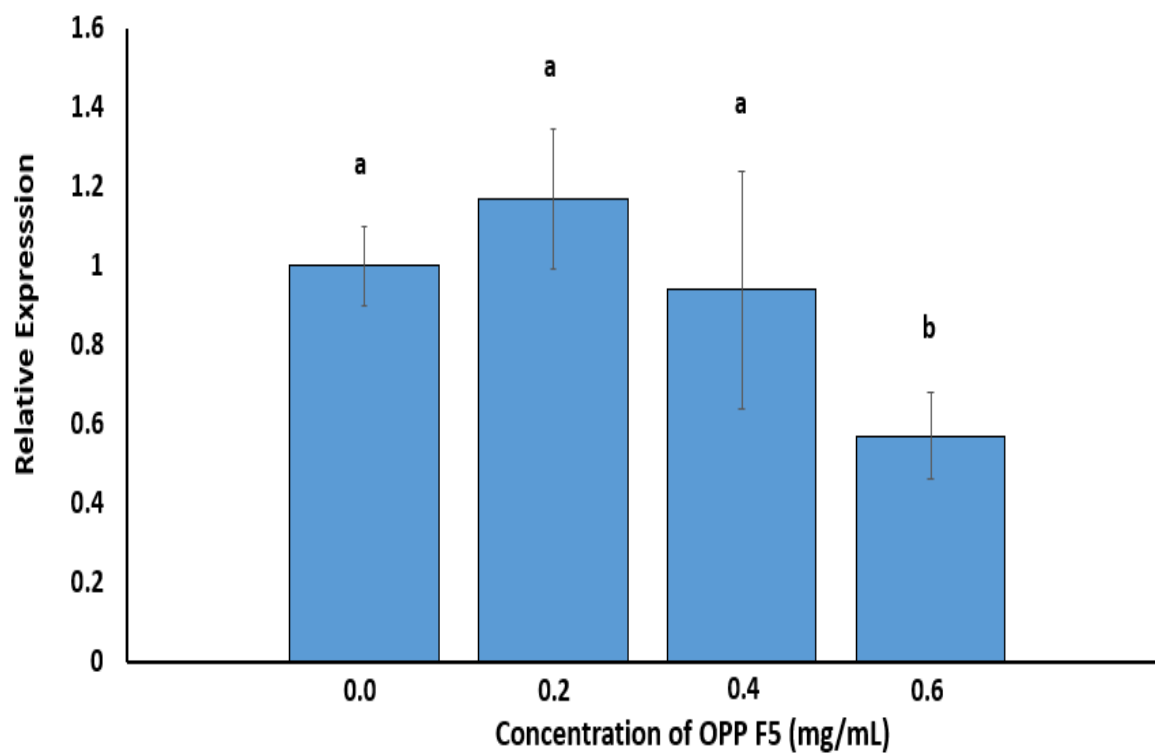
Data is represented as mean ± S.E.M. Lowercase manuscript letters [a, b, c] **P < 0.05**, represents differences between the treatments in relation to control (0), and analysis was conducted using Independent t-test comparison, and One-way ANOVA.
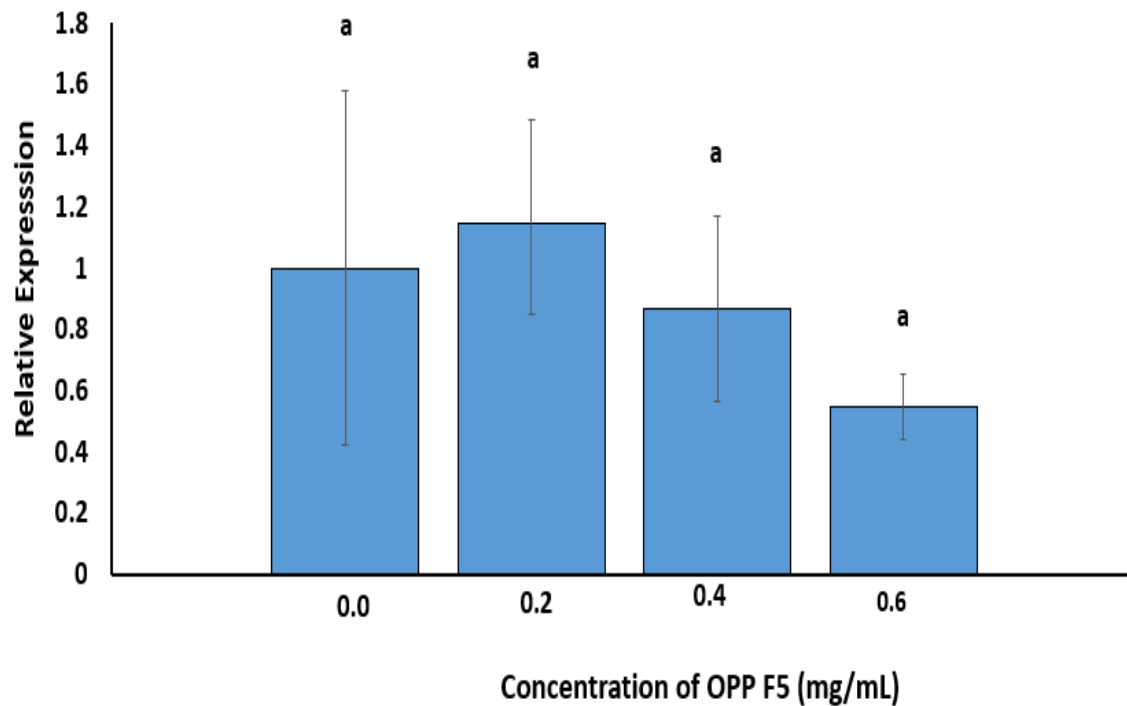
Figure 3-11. MTOR Expression: PANC-1 Cell Line

Data is represented as mean ± S.E.M. Lowercase manuscript letters [a, b, c] **P < 0.05**, represents differences between the treatments in relation to control (0), and analysis was conducted using Independent t-test comparison, and One-way ANOVA.

Figure 3-12. MTOR Expression: BxPC-3 Cell Line

Data is represented as mean ± S.E.M. Lowercase manuscript letters [a, b, c] **P < 0.05**, represents differences between the treatments in relation to control (0), and analysis was conducted using Independent t-test comparison, and One-way ANOVA.

CHAPTER 4: DISCUSSION

One of the major causes of cancer-related deaths in the United States is pancreatic cancer (PACA). Its prognosis is poor; at 8% five-year survival rate, which is mostly due to lack of effective treatment and failure to detect it early due to lack of effective detection methods [65, 66]. Among patients with nonresectable PACA, Gemcitabine, a deoxycytidine analog, has been the drug of choice. It is genotoxic and considered an antimetabolite [67].

Antimetabolites are similar in structure with normal compounds required for normal biochemical activity but in differ in that they can interrupt normal cell function. One of the most important mechanisms through which gemcitabine work is through the inhibition of DNA synthesis, which makes it the preferable chemotherapeutic agent [68]. A single integration of deoxynucleotide, once gemcitabine is activated in a cell, stops chain elongation [69]. Although gemcitabine is the first line of treatment for PACA, this therapy is challenging considering PACA can be aggressive, chemoresistant and the drug may have adverse side effects. Consequently, it is of vital importance to develop new treatment modalities for PACA that are relatively non-toxic, either s single agents or a combination with other drug treatments. Several researchers have investigated and documented the anticancer properties of several phytochemicals such as curcumin and garcinol [70, 71], lycopene [72, 73], resveratrol [74], ECGC or epigallocatechin gallate [75] and genistein [76, 77].

Oil palm (Elais guineesis) in the family of Arecaceae is a high oil plant, whose water-soluble materials are extracted to produce phytochemicals such as phenolics and organic acids, which are collectively known as Oil Palm Phenolics (OPP) [78]. OPP contains high levels of antioxidants, which our lab's recent in vitro work has shown to have therapeutic effects against PACA [79]. In this study, panc1 and BxPC-3 PACA cell line models were used to investigate the

effect of OPP, a potential chemotherapeutic compound, on PACA treatment. We report that administering dietary OPP revealed a dose-depended reduction in the growth and proliferation of both BxPC-3 and Panc1 cells. There was a dose-dependent anti-invasive effect that OPP showed in Panc1 and BXP3 cells with concentration levels ranging between 0 and 0.8 μg/ml in Matril gel invasion assay (Fig. 3-4 & 3-5). The ability of OPP to inhibit the migration of cells after a 30-hour treatment session with and 0.8 μg/ml was significant. In addition, OPP exhibited significant inhibition of the DNA-binding activity of NF-κB, which was also dose-dependent for both cell lines (Fig. 3-8). Also, there was a dose-dependent reduction in the expression of the BCL2 gene as shown in (Fig. 3-9). Previous in vitro work on OPP showed that the anticancer potential of OPP occurred through the phenolics' ability to suppress the NF-κB pathway with MMP9 cancer-related marker's favorable regulation along with other markers [79].

In this study, we examined the impact of OPP on BCL2, a protein-coding gene that regulates apoptosis [80]. In addition, we evaluated the downstream gene mTOR expression. The downstream gene effector is involved in the survival, growth, proliferation, and mobility of cancer cells and apoptosis [81, 82]. The study shows that the cells treated with OPP have significantly lowered the expression of BCL2 (Fig. 3-10) and mTOR (Fig. 3-11 & 3-12). Inhibiting mTOR signaling leads to a reduction in cell growth and protein synthesis. These findings support the hypothesis that OPPs have anticancer activity and are capable of inducing apoptosis and inhibiting growth through interrupting BCL2 and mTOR activity when used as a single agent.

Taken together, this study has led us to conclude that oil palm phenolics (OPP) demonstrates anticancer activities when used in vitro pancreatic cancer PANC-1 and BXPC-3 cell lines. Findings from this study can be further investigated at a larger scale in an animal study which may potentially lead to a pilot study. Clinical studies on nutraceuticals and their effect on the

pathways involved in preventing cancer development is lacking yet many cancer patients turn to nutraceuticals because they believe they may benefit from their clinical outcome without experiencing toxic side effects. There is future hope, in that in vivo experiments with OPP have inhibited some aspects of cancer development. The long-term plan is to identify specific treatment targets of OPP, in order to improve the general quality of life and extend the survival rate of pancreatic cancer patients.

A follow-up study can be conducted to determine the active molecular components in OPP fraction 5. OPP fraction 5 can be separated by reversed-phase HPLC can be used for reversed-phase separation, and peaks can be further analyzed by MS and NMR spectroscopy. There is limited data available on the appropriate dosage needed to determine OPP fraction 5 anti-cancer effect in a clinical trial. However, a phase one 1 clinical study, OPP Dosage was chosen according to the determination of pomegranate juice's GAE intake, showed that ingesting 1420/mg/ day of dietary pomegranate supplementation is safe in healthy human volunteers (870mg GAE/DAY TOTAL). In another study, a total of 450 mg GAE/DAY of OPP was given to 60 volunteers for 60 days [84]. The data from this study can be utilized to translate an effective dose to be used in pre-clinical trials on animal models treated with OPP fraction-5.

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ABSTRACT

THE EFFECT OF OIL PALM PHENOLICS (OPP) ON PANCREATIC CANCER CELL LINES

by

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Major: Nutrition and Food Science

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Pancreatic cancer (paca) is currently the fifth causes of cancer-related deaths in the United States. It's an aggressive form of cancer with very low survival rates because of delayed diagnosis and limited treatment options. Gemcitabine is the chemotherapy drug that provides minimal benefits along with many side effects. The aim of this study was to examine the in vitro effects of oil palm phenolics (OPP) fraction-5, the water-soluble component of palm oil, in human pancreatic cancer cell models. Two pancreatic cancer cell lines (Panc-1 and BxPC-3) were categorized into control and treatment groups. The control group received cell culture media and the treatment groups received different concentrations of OPP. A statistically significant $P<0.05$) dose-dependent decrease in cell viability was observed in both cell lines after the addition of OPP by MTS assay.

Furthermore, a significant decrease in cell invasion and migration was seen in both cell lines when compared to untreated controls. Because OPP showed a decrease in cell proliferation, invasion, and migration, RT-PCR was used to determine the molecular pathways behind the above observations by testing the expression of both the mTOR and BCL-2 genes. A dose-dependent

decrease was observed in MTOR AND BCL-2 proteins. Also, OPP showed an inhibition of the NF-KB pathway.

Thus, the data in this study signifies the role of OPP as a potential therapeutic agent against the progression of pancreatic cancer and warrants further studies to investigate OPP as a potential safe, natural therapeutic agent to treat PACA.

AUTOBIOGRAPHICAL STATEMENT

Education:

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